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Physiques that perform: The interaction between body composition management, performance and calcium homeostasis in female cyclists

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ABSTRACT

Body composition of female cyclists is believed to have an impact on performance. The central aim of this thesis was to address social, technical and health related issues around body composition management in this population. The first study (Chapter 3) examined the satisfaction of elite female cyclists with their body weight in the context of race performance, the magnitude of body weight manipulation and the association of these variables with menstrual function. Female competitors in the Australian National Road Cycling Championships ($n = 32$) and the Oceania Championships ($n = 5$) completed a questionnaire designed to identify attitudes towards body weight as it relates to performance, the magnitude of body weight change and the techniques used to manipulate body weight. All but one cyclist reported that female cyclists are a *weight conscious population* and 54% reported having a desire to change body weight at least once weekly. Of the cyclists surveyed, 62% reported that their current body weight was not ideal for performance. The perceived ideal body weight was (mean \pm SD) 1.6 ± 1.6 kg ($2.5 \pm 2.5\%$) less than their current weight and 73% reported their career lowest body weight was either '*beneficial*' or '*extremely beneficial*' for performance. A majority (65%) of the cyclists reported successfully reducing body weight in the previous 12 mo with a mean loss of 2.4 ± 1.0 kg ($4.1 \pm 1.9\%$). The most common weight loss technique was reduced energy intake (76%). Five cyclists (14%) had been previously diagnosed as having an eating disorder by a physician. Of the 18 athletes not using a hormonal contraceptive, 11 reported menstrual dysfunction (oligomenorrhea or amenorrhea). The first study showed that elite female cyclists are a weight conscious population who may not be satisfied with their body weight leading into a major competition and in some cases, are frequently weight conscious.

The purpose of the second study (Chapter 4) was to describe normative values and seasonal changes for body composition in female road and track endurance cyclists. During a 16 y period, anthropometric profiles ($n = 616$) were measured from 126 cyclists categorised as *World class*, *Elite* and *Sub-Elite*. The date of each profile measurement was categorised to a season phase: *Off-Season* (October 1 – December 31); *Early-Season* (January 1 – April 30) and *Late-Season* (May 1 – September 30). Compared to *Sub-Elite* and *Elite* road cyclists, *World class* road cyclists were on average (Mean [95 % CI]) 1.18 kg [0.46, 1.90] and 0.60 kg [0.05, 1.15] lighter and had skinfolds that were 7.4

mm [3.8, 11.0] and 4.6 mm [1.8, 7.4] lower respectively. There were main effects for season phase with higher values measured for body weight (0.41 kg [0.04, 0.77]) and skinfolds (4.0 mm [2.1, 6.0]) in the *Off-Season* compared to the *Early-Season*. Compared to *Track Endurance* cyclists at the *World Class* level, female *Road* cyclists had lower body weight (6.04 kg [2.73, 9.35]) and skinfolds (11.5 mm [1.1, 21.9]). Study 2 concluded that higher performing female road cyclists are lighter and leaner than their less successful peers as well as track endurance cyclists.

While a low body weight is a common goal of road cyclists, little is known about the relationship between functional lean mass and power output. In study three (Chapter 5), amateur female road cyclists ($n = 33$) performed a power profile (6, 15, 30, 60, 240, s maximal effort) on a wind-braked ergometer. Maximal mean power (MMP) for each bout was compared to lower body lean mass (LBLM) measured using dual energy X-ray absorptiometry (DXA). The MMP for efforts of all durations were significantly correlated with LBLM. Relative to the durations of the effort, the slope of the relationship reduced in a curvilinear fashion indicating that the contribution of LBLM to power output for efforts greater than 240 s is stable at ~ 10 W/kg LBLM. For shorter durations, the slope was greater: MMP 1 s, 64.6 W/kg LBLM ($R^2 = 0.64$); MMP 5 s, 59.5 W/kg LBLM ($R^2 = 0.65$); MMP 15 s, 40.5 W/kg LBLM ($R^2 = 0.50$). The study showed that LBLM explains a moderate-to-high proportion of maximal cycling power output for efforts lasting less than 2 min in duration.

In order to better understand changes in energy balance (EB) when body composition is being manipulated, measures of energy intake (EI) as well as energy expenditure (EE) require accurate measurement. Study four (Chapter 6) validated the use of commercially available power meters (Schoberer Resistance Measurement, Jülich, Germany; SRM) for estimating EE during variable intensity cycling. Female road cyclists ($n = 9$) completed a gross efficiency test ($GE_{\text{test}} = 4$ min at $\sim 45\%$, $\sim 55\%$, $\sim 65\%$ and $\sim 75\%$ maximal aerobic power; MAP) before and after 10.5 min of either constant (CON) or variable (VAR) intensity cycling averaging $\sim 55\%$ MAP. Gross efficiency (GE) measured pre-, post- and during CON and VAR cycling was compared. Total EE for 10.5 min of VAR cycling was estimated using indirect calorimetry (CAL) and compared to estimates based on mechanical power (SRM) using each athlete's mean GE (GE_{IND}). There was no effect of VAR on GE_{tests} . The difference between GE (mean \pm SD) measured during CON ($18.4 \pm 1.6\%$) and VAR cycling ($18.6 \pm 1.1\%$) was trivial. SRM based estimates of EE using

each athlete's mean GE were all <2% of CAL. The findings of this study support the use of calibrated power meters for estimating cycling EE during variable intensity cycling <75% MAP.

Study five (Chapter 7) presents a case study of a female cyclist returning to elite competition following a period of post-viral chronic fatigue associated with poor weight management. Body composition was measured using DXA and anthropometry. Dietary manipulation involved a modest reduction in energy availability (EA) to 30 - 40 kcal / kg / fat free mass / d and an increased intake of high quality protein, particularly following training (~20 g). Through the re-training period, total body weight decreased (-2.82 kg), lean mass increased (+0.88 kg) and fat mass decreased (-3.47 kg). Favourable body composition changes and training adaptations were achieved through a subtle energy restriction associated with increased protein intake and sufficient EI during training.

Being weight conscious and having a desire to optimise power output relative to weight, exposes female cyclists to an increased risk of poor bone health. The final two studies (Chapters 8 & 9) investigate the effects of a pre-exercise calcium supplementation via a meal on cycling performance, gut comfort and biomarkers of bone turnover to consider the effects of this strategy in maintaining bone health. Well-trained female cyclists (n = 32) completed two 90 min cycling trials (80 min at 60% MAP followed by a 10 min time trial) separated by 1 day. Exercise trials were preceded by 2 h with either a calcium-rich (1352 ± 53 mg calcium) dairy based meal (CAL) or a control meal (CON; 46 ± 7 mg calcium). Blood was sampled pre-trial; pre-exercise; and immediately, 40 min, 100 min and 190 min post-exercise. Blood was analysed for ionized calcium (iCa) and biomarkers of bone resorption: Cross Linked C-Telopeptide of Type I Collagen (CTX-I) and Parathyroid Hormone (PTH) using the enzyme-linked immunosorbent assay (ELISA) technique. Parathyroid Hormone and CTX-I concentrations increased from pre-exercise to post-exercise in both conditions but were attenuated in CAL. Parathyroid Hormone was 1.55 [1.20, 2.01] times lower in CAL immediately post-exercise and 1.45 [1.12, 1.88] times lower at 40 min post-exercise. Cross Linked C-Telopeptide of Type I Collagen was 1.40 [1.15, 1.70] times lower in CAL immediately post-exercise, 1.30 [1.07 – 1.57] times lower at 40 min post-exercise and 1.22 [1.00, 1.48] times lower at 190 min post-exercise. There was no effect of meal type on measures of time trial performance or gut comfort. The investigation found that a calcium-rich pre-exercise breakfast meal containing ~1350 mg of

calcium consumed ~90 min before a prolonged and high intensity bout of stationary cycling attenuates the exercise induced rise in markers of bone resorption.

In conclusion, this thesis represents a unique collection of studies that investigate a broad range of themes around body composition in female cyclists. The findings provide insight into body composition performance relationships, how body composition might be manipulated and a potential strategy to better maintain bone health. The data support and extend research which has been performed with male cyclists and provides practical tools and strategies which can be realistically implemented by female endurance athletes and sport scientists in the daily training environment.

DECLARATION BY AUTHOR

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

I have clearly stated the contribution of others to my thesis as a whole, including statistical assistance, survey design, data analysis, significant technical procedures, professional editorial advice, and any other original research work used or reported in my thesis. The content of my thesis is the result of work I have carried out since the commencement of my research higher degree candidature and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution. I have clearly stated which parts of my thesis, if any, have been submitted to qualify for another award.

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PUBLICATIONS DURING CANDIDATURE

Haakonssen, E.C., Martin, D.T., Burke, L.M., Jenkins, D.G. Energy expenditure of constant- and variable-intensity cycling: power meter estimates. *Medicine and Science in Sports and Exercise*. 2013 Sep;45(9):1833-40. doi: 10.1249/MSS.0b013e31828e18e6.

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Conference abstracts & presentations

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Haakonssen, E.C., Martin, D.T., Burke, L.M., Jenkins, D.G. Lean mass - cycling power relationships in female road cyclists. *American College of Sports Science World Congress 2013* – Abstract and poster presentation.

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Jenkins DG	Wrote and edited the paper (10%)
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 - Research design, data interpretation, editing written work
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**STATEMENT OF PARTS OF THE THESIS SUBMITTED TO QUALIFY FOR
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None

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LIST OF ABBREVIATIONS

ADP	Air displacement plethysmography
BIA	Bioimpedance analysis
BMR	Basal metabolic rate
BSA	Body surface area
BW	Body weight
CHO	Carbohydrate
CSA	Cross-sectional area
CT	Computed tomography
CTX	Cross linked C-telopeptide
BD	Body density
DLW	Doubly labelled water
DXA	Dual energy x-ray absorptiometry
EA	Energy availability
EB	Energy balance
ECW	Extracellular water
ED	Energy density
EE	Energy expenditure
EI	Energy intake
ELISA	Enzyme-linked immunosorbent assay
FA	Frontal area
FFM	Fat free mass
FFQ	Food frequency questionnaire
FM	Fat mass
GE	Gross efficiency
GI	Gastrointestinal
GPS	Global positioning system
GTRH	Gonadotropic releasing hormone
HEF	High energy flux
HR	Heart rate
ICW	Intracellular water
IOC	International Olympic Committee
LBLM	Lower body lean mass

LEF	Low energy flux
MAP	Maximal aerobic power
MMP	Maximal mean power
MRI	Magnetic resonance imaging
NTX	Amino-terminal cross-linking telopeptide
OC	Osteocalcin
PAL	Physical activity level
PRO	Protein
PTH	Parathyroid hormone
RDA	Recommended daily allowance
RED-S	Relative energy deficiency in sport
RIA	Radioimmunoassay
RPE	Rate of perceived exertion
RQ	Respiratory quotient
TBW	Total body water
TEF	Thermic effect of food
UCI	Union Cycliste Internationale
USG	Urine specific gravity
VAS	Visual analogue scale
$\dot{V}O_2$	Oxygen consumption
$\dot{V}CO_2$	Carbon dioxide expired

CHAPTER 1: INTRODUCTION

1.1 Thesis overview

In endurance sports such as distance running and road cycling, a low body weight (BW) and low body fat content are believed to be important for performance (Sundgot-Borgen et al., 2013). This thesis will focus on better describing the body composition of elite and world class female cyclists so that coaches and athletes can understand what is ideal but also what can realistically be achieved. Once the athlete has a clearly defined goal, there is a need for experimental data to guide body composition manipulation strategies. In addition to performance goals related to body composition, health concerns which stem from weight manipulation in this weight sensitive sport must be considered. Female cyclists have been identified as a population at risk of low bone mineral density (Sherk et al., 2014). A secondary focus of this thesis will be on the integration of nutritional strategies to help maintain calcium homeostasis and bone health.

Seven studies were conducted to investigate different aspects of body composition in female road cyclists. While there is a general perception within the cycling community that road cyclists are a weight conscious population who frequently manipulate their weight to improve performance, these themes have not received robust scientific attention particularly in female road cyclists. Furthermore, few data have been published describing the relationships between body composition and performance as well as seasonal changes in body composition. The first study examined the satisfaction of elite female cyclists with their BW in the context of race performance, the magnitude of BW manipulation and the association of these variables with menstrual function. Female competitors in the Australian National Road Cycling Championships and the Oceania Championships completed a questionnaire designed to identify attitudes towards BW as it relates to performance, the magnitude of BW change and the techniques used to manipulate BW. Results from the first study suggested that elite female cyclists are a weight conscious population who are often not satisfied with their BW leading into a major competition.

Given the finding that female cyclists perceive that being light and lean is important for performance, the second study was designed to describe normative values and seasonal changes in body composition in female cyclists. Over 16 y, a large number of

anthropometric profiles were measured from cyclists categorised as *World class*, *Elite* and *Sub-Elite*. The results from this study suggest that higher performing female road cyclists are lighter and leaner than their less successful team mates and that there is margin of seasonal variation with higher measures for BW and skinfolds in the off-season compared to during the competitive season.

Given that much of the focus appears to be on reducing BW as opposed to manipulating body composition, the terms *functional* and *non-functional mass* were introduced. *Functional mass* describes mass that contributes directly towards performance whereas *non-functional mass* includes fat mass and lean mass that may hinder performance e.g. upper-body lean mass for road cyclists. While having a low BW appears to be important, cyclists must also have sufficient *functional mass* (lower body lean mass; LBLM) to produce the required power output. Little is known about the relationship between functional lean mass and power output. Therefore, study three compared measures of LBLM using dual energy x-ray absorptiometry to maximal mean power (MMP) for efforts of increasing duration (6, 15, 30, 60, 240, s) performed on a wind-braked ergometer by amateur female cyclists. The MMP for efforts of all durations were significantly correlated with LBLM. Relative to the duration of the effort, the slope of the relationship reduced in a curvilinear fashion, indicating that the contribution of LBLM to power outputs for efforts greater than 240 s is stable at ~10 W/kg LBLM. Lower body lean mass explained a moderate-to-high proportion of maximal cycling power output for efforts lasting less than 2 min in duration, so should possibly be preserved during weight loss practices. These findings also allow the coach and sport scientist to determine whether an athlete is producing the predicted power output for a given LBLM (muscle quality). Substantial deviations from the expected relationship may guide interventions to either focus on increasing LBLM or improving muscle recruitment and force development through other forms of training.

In order to manipulate body composition, an understanding of energy intake (EI) in relation to energy expenditure (EE) is important to accurately establish an athlete's energy availability (EA). The fourth study validates the use of commercially available power meters (SRM) for estimating EE during variable intensity cycling. Australian National Team female road cyclists completed a gross efficiency (GE) test before and after a bout of either constant or variable intensity cycling resulting in the same total work completed. The results showed that SRM based estimates of EE using each athlete's mean GE were

all within <2% of estimates calculated using indirect calorimetry. These findings support the use of calibrated power meters for estimating cycling EE during variable intensity cycling <75% of maximal aerobic power output. This work rate is representative of the majority of cycling training and much of race performance.

Study five provides an example of how tools such as anthropometry, DXA and power meters can be used to guide and monitor a body composition manipulation strategy. This study presents a case of a female cyclist returning to elite competition following a period of post-viral chronic fatigue associated with poor weight management. The outcome of this intervention showed that total BW can be decreased while lean mass is increased and favourable training adaptations occur. Briefly, the intervention created a subtle energy restriction associated with increased protein intake and sufficient EI timed to support a training program which included strength based training.

There is compelling evidence that bone health may be compromised by energy restriction (Loucks et al., 2011), participation in sports with high volumes of non-weight bearing endurance training (Scofield and Hecht, 2012) and, more recently, exercise induced sweat calcium losses (Barry et al., 2011; Guillemant et al., 2004). In addition to being weight conscious and having a desire to optimise power output relative to BW, female cyclists have a high prevalence of poor bone health (Sherk et al., 2014). Previous investigations (Barry et al., 2011; Guillemant et al., 2004) found that ingestion of calcium-fortified water before and during cycling attenuates exercise induced perturbations in calcium homeostasis and biomarkers of bone resorption. Strategies that could help to preserve bone health, support training and complement strategies to manipulate body composition could be valuable. Dairy protein is proposed as having a high-biological value, accelerating weight loss and preserving lean mass (Phillips and Zemel, 2011; Zemel, 2004). Consuming a high calcium dairy-rich meal may attenuate bone resorption during cycling and help athletes meet other nutritional guidelines. The last two studies compare the effects of a calcium-rich dairy based pre-exercise meal on biomarkers of bone turnover. The results suggest that a calcium-rich pre-exercise breakfast meal consumed before a prolonged and high intensity bout of stationary cycling attenuates the exercise induced rise in biomarkers of bone resorption without causing gut discomfort or impairing performance when compared to a control meal.

Collectively, this series of studies investigates a broad range of themes around body composition in female cyclists. The data provide insight into body composition performance relationships, how body composition might be manipulated and a potential strategy to better maintain bone health. The findings support and extend research which has been performed with male cyclists and provides practical tools and strategies which can realistically be implemented by athletes and sport scientists in the daily training environment.

1.2 General introduction

A key performance indicator for both male and female cyclists is the maximal amount of power that can be generated relative to BW. Both physical training and diet interact to influence the cyclist's BW, body composition and their ability to produce power over different durations. Over the course of an athlete's development, it is common that a physiological ceiling will be reached in terms of the maximum amount of power that can be produced over given exercise durations. In these cases, athletes may manipulate their BW to improve their power-to-weight ratio.

Female road cyclists compete in a range of different types of races, from one-day races through to multi-day stage races. Individual races range from individual time trials where each cyclist completes the course individually and the athlete with the fastest time wins. Mass start races form the bulk of road races and these vary in distance and profile and can last up to ~140km or >4 h. In these races, all riders start at the same time and the winner is the cyclist who completes the course first. In stage races, the cumulative time to complete each stage is used to determine the ranking of riders and the cyclist who completes all stages in the least amount of time will win the overall classification. Due to the different types of terrain and the different styles of racing, riders with different morphologies and of varying physiology are often suited to some race courses more than others. Riders are generally classified as specialists in climbing, sprinting or time trial / flat land, with some being classified as all-rounders. Work by Impellizzeri et al. (2008) describes the relationship between morphological characteristics (BW, body surface area, frontal area) and cycling performance. These authors showed that absolute physiological parameters were higher in time trial specialists compared to climbers. When normalised for BW, the same parameters were similar between climbers and time trialists (Impellizzeri et al., 2008).

While cycling is an individual sport, it is often raced in teams where riders will take advantage of their riders' strengths and exploit the opposition's weaknesses to put their team leader in the best possible position to win. The highly strategic nature of road cycling is made possible largely by the fact that aerodynamic drag and therefore the power required for a given speed is reduced substantially when drafting behind another rider or when sheltered within a bunch (*peloton*) of many cyclists (Lukes et al., 2005; Olds et al., 1995).

Over the last 20 y, power output measuring tools such as the SRM power meter which enable the quantification of mechanical work in the field and the laboratory have become commercially available. Using power meters in combination with other techniques, Martin et al. (2001) published data detailing the physiological characteristics of female road cyclists while Ebert et al. (2005) have described the power output demands of World Cup top twenty finishers. These studies provide great insight into the demands of competition and performance characteristics of female road cyclists. In general however, there is very little research involving female cyclists. Specifically, there is a large gap in the literature addressing themes related to body composition management in this population.

The major themes surrounding body composition maintenance and manipulation involve the accurate and reliable measurement of body composition, accurate and reliable measurement of energy availability (energy intake less exercise energy expenditure) and the creation of combined nutritional and training strategies that are safe, effective and tolerable by athletes. This thesis will begin to address some of these themes as they relate to female road cyclists.

CHAPTER 2: REVIEW OF THE LITERATURE

2.1 Introduction

There is a logical relationship between power output and body weight (BW) in road cycling, where for a given power output a cyclist with a lower BW will accelerate and climb faster than a heavier competitor. More than 13 years ago, Martin et al. (2001), published normative values for physiological characteristics of a small sample of Australian National Team female road cyclists. This included lab and field based performance testing and basic anthropometry. The higher performing cyclists in this group were lighter and leaner than their team mates who performed less well. Likewise, Impellizzeri et al. (2008) have described clear relationships between the morphology of female cyclists and their uphill cycling performance. It is the experience of coaches and scientists at the Australian Institute of Sport, that road cyclists are athletes who typically present with a low BW and low body fat mass. Despite the apparent importance of BW and morphology for road cycling performance, little research exists to describe the interaction between body composition and performance, how it can be manipulated and strategies that can minimise the risk of poor health outcomes. This chapter will review aspects of body composition in the context of athletic performance and health.

The first section of this review of the literature will briefly describe the sport of women's road cycling. The second section will focus on the measurement of body composition and the various techniques available. Following on from this, the review will discuss techniques used to measure both energy intake (EI) and energy expenditure (EE). The final section of this chapter will describe the body's physiological response to acute and chronic energy deficits. This will include the possible interaction between low energy availability (EA), participation in high level road cycling and bone health. Some of the techniques used to measure these relationships will be described.

2.2 The demands of competition in women's road cycling

Racing formats for women's road cycling range from single-day events through to multi-day stage races and *Grand Tours* such as the *Giro d'Italia Femminile Internazionale* which can last up to 10 d (see Table 1). Included in the single-day events are the One-Day Spring Classics such as La Flèche Wallonne and the Tour of Flanders; the Olympic

Games road race and the World Championship road race held annually. For women, these races cover distances of up to 170 km and can last for up to five hours although more commonly they are <140 km.

Table 1. Characteristics of typical women's road cycling competitions

	<i>Race Format</i>	<i>Examples</i>	<i>Typical distance (duration)</i>
<i>Single-day events</i>	Mass-start road race	<i>La Flèche Wallonne</i> <i>Tour of Flanders</i> <i>World Championship</i> <i>Olympic Games</i>	60 - 170 km (2 - 5 hr)
	Individual time trial	<i>World Championship</i> <i>Olympic Games</i>	20 - 35 km (30 - 50 min)
	Teams time trial	<i>World Championship</i>	30 - 40 km
<i>Stage races</i>	3 - 5 d with daily program including: • Mass-start road races • Individual time-trials • Teams time trials	<i>Giro del Trino</i> <i>Tour de l'Ardeche</i>	<i>A combination of the above</i> Some include a prologue (short individual time trial <10 km)
	6 - 10 d with daily program including: • Mass-start road races • Individual time-trials • Teams time trials	<i>Giro della Toscana</i> <i>Giro d'Italia</i> <i>Route De France</i>	<i>A combination of the above</i> Some include a prologue (short individual time trial <10 km)

Women's professional cycling has experienced a dramatic increase in popularity and competitiveness in the last decade. It was only in 1998 that cycling's governing body, the Union Cycliste Internationale (UCI) started the Women's Road Cycling World Cup; a season-long competition consisting of 6 - 12 races for which points are awarded to give an overall ranking system. The format of races has also become increasingly challenging. Prior to 1996, the Women's World Championship Road Race had never exceeded 100 km in length while the typical format since 2010 has been ~125 km. Furthermore, it was not until 1994 that the UCI began to honour the Women's Time Trial as a World Championship event. The professional scene has been accompanied by greater financial backing as a result of increased popularity. In 2000, there were only 17 professional women's teams registered with the UCI, while in 2014 there were 27. With this increased popularity, the sport has become much more competitive and so athletes, coaches and sports scientists are encouraged to seek greater performance gains.

2.3 The interaction between body weight and power output in women's road cycling

In cycling, power output is now easily measured in the field. Power meters such as *SRM* (Schoberer Resistance Measurement, Jülich, Germany), *Quarq* (Quarq Technology, Spearfish,, SD, USA), *Power Tap* (CycleOps, Madison, WI, USA) and *Vector Pedal* (Garmin, Olathe, KS, USA) are crank, wheel hub or pedal-based systems respectively. These devices measure the mechanical power output produced by the cyclist by multiplying torque applied to the crank, wheel hub or pedal spindle by the angular velocity of these components. The application of torque causes the component to experience slight deformation, which is measured by strain gauges within the power meter. The power meter converts the torque into a high frequency electrical signal that along with cadence is sent to a handlebar-mounted computer which calculates power output in watts using Equation 1 (Schoberer, 2014).

Equation 1

$$\text{Power output} = \frac{([\text{measured frequency} - \text{zero offset frequency}] \cdot \text{cadence} \cdot 2\pi)}{(\text{slope} \cdot 60)}$$

2.4 Specialisation of female cyclists

The body dimensions (morphology) and physiology associated with level ground and uphill cycling performance in female cyclists has been previously reviewed. Impellizzeri et al. (2008) described the appearance of morphological characteristics that dictate the specialization of these athletes into climbing, flat terrain and time trial specialists. Flat terrain specialists (mean \pm SD; 58.0 \pm 4.6 kg) and time trialists (61.6 \pm 3.1 kg) had higher BW, body surface area (BSA), and anterior projected frontal area (FA) than climbers (51.8 \pm 3.4 kg). The BSA and FA of flat terrain cyclists and time trialists were lower than climbers when expressed relative to BW. This provides an aerodynamic advantage on the flat.

While flat terrain cyclists and time trialists may be heavier, their mass provides an advantage only when it constitutes functional mass which can be regarded as lower body lean mass. Additional fat mass or excessive non-functional lean mass (e.g. upper body

lean mass) will only burden a rider by increasing BW, BSA and FA. While different morphological characteristics lend themselves to specialisation, there is a well-accepted performance benefit associated with being as light and lean as possible, so long as health and sustainable power output are unaffected.

2.4.1 Climbing

Often the capacity of a cyclist to hold a given power output for a given time is expressed relative to BW (W/kg). A high power to weight ratio is paramount when it comes to climbing ability. Over the last decade, every Women's World Championship road course has included at least one significant climb, which was repeated on a circuit ~6 to 10 times (see Table 2). The longest and most prestigious *Grand Tour* for women is the *Giro d'Italia Femminile Internazionale*; an annual race which includes mountain passes such as Italy's Mortirolo; a 12.4 km climb with an average gradient of 10.5% (maximum 18%) and a total elevation gain of 1300 m. It is clear that with first principles of physics in mind, a low BW is favourable for tackling hilly terrain. Padilla et al. (1999) have demonstrated that flat terrain specialists display the highest absolute maximal aerobic power (MAP) while uphill specialists display the highest MAP relative to their BW. Furthermore, Mujika and Padilla (2001) have shown that male uphill specialists are shorter and lighter than flat terrain specialists, which is similar to findings for female cyclists (Impellizzeri et al., 2008).

Careful analysis of power data has revealed power outputs required to perform at World Cup races. Ebert et al. (2005) have shown that in hilly races, top 20 finishers in Women's World Cup races produced an average (\pm SD) power of 3.0 ± 0.4 W/kg for durations of 187 ± 19 min. Cyclists produced Maximal Mean Power (MMP) values of 5.7 ± 0.6 , 5.6 ± 0.4 and 5.3 ± 0.4 W/kg for 180, 240 and 300 s, respectively. In short, it is a prerequisite for female cyclists to be able to sustain ~5.5 W/kg for more than 4 min to be competitive in hilly world cup races.

Table 2. Summary of UCI women's road cycling World Championship courses from 2004 – 2013

<i>Year</i>	<i>Location</i>	<i>Climbs: distance, average gradient, number of circuits</i>	<i>Race distance (km)</i>
2014	Ponferrada, Spain	Climb 1: 7.2 km, 2.7 % Climb 2: 1.92 km, 3.3 % 18.2 km circuit repeated 7 times	127.4
2013	Tuscany, Italy	Climb 1: 4.4 km, 5.2 % Climb 2: 0.6 km, 10.2 % 16.6 km circuit repeated 5 times	139.7
2012	Limburg, Netherlands	Climb 1: 1.3 km, 3.0 % Climb 2: 1.5 km, 4.7 % 16.1 km circuit repeated 8 times	129.0
2011	Copenhagen, Denmark	Climb 1: 0.8 km, 4.0% 14.0 km circuit repeated 10 times	140.0
2010	Geelong & Melbourne, Australia	Climb 1: 1.72 km, 4.6 % Climb 2: 1.14 km, 2.3 % 15.8km circuit repeated 8 times	127.2
2009	Mendrisio, Switzerland	Climb 1: 2.14 km, 4.6 % Climb 2: 2.40 km, 4.8% 13.8km circuit repeated 9 times	124.2
2008	Varese, Italy	Climb 1: 1.48 km, 3.1 % Climb 2: 3.55 km, 3.7 % 17.4km circuit repeated 8 times	138.8
2007	Stuttgart, Germany	Climb 1: 1.87 km, 3.2% Climb 2: 1.73 km, 3.4% Climb 3: 2.28 km, 2.3 % 19.1km circuit repeated 7 times	133.7
2006	Salzburg, Austria	Climb 1: 2.63 km, 3.0 % 22.2km circuit repeated 6 times	132.6
2005	Madrid, Spain	2 Climbs 21.0km circuit repeated 6	126.0
2004	Verona, Italy	1 Climb 14.0km circuit repeated 9 times	132.8

Union Cycliste Internationale (UCI)

2.4.2 Accelerating and sprinting

Climbing is not the only aspect of cycling in which BW impacts performance. Being able to accelerate rapidly is necessary when sprinting on both flat and hilly terrain. Accelerations take place when initiating or responding to attacks, coming out of turns and challenging sprints. Newton's 2nd law states that acceleration is equal to the sum of forces applied to an object divided by its mass. For a given force application, acceleration is inversely related to an object's mass. Being light and powerful improves the cyclist's ability to execute rapid accelerations.

Ebert et al. (2006) have reported relatively low average power outputs during professional men's races, although they are characterised by many accelerations. Male cyclists complete ~70, 40 and 20 *sprints* (power outputs greater than MAP) during criterium, hilly and flat races respectively. While a similar analysis has not been reported for female cyclists, it is known that in flat World Cup races, top 20 finishers accumulate 12.4 ± 4.9 min above 7.5 W/kg in races lasting just over two hours (Ebert et al., 2005). It is likely that in women's road cycling, the frequency and distribution of accelerations is similar to that of men's.

2.4.3 Time trialling and solo efforts

Climbing and accelerating are both key components of a successful cyclist's repertoire. It is also important that competitive cyclists can sustain power outputs that represent a high percentage of their MAP for prolonged (>10 min) periods. This is the underpinning characteristic of a time trialist or a one-day race specialist, who can ride solo or in small groups (*breaks*) off the front of the main bunch (*peloton*) of cyclists.

The conversion of cycling power output to cycling speed has been characterised by mathematical models. Olds et al. (1995) published a mathematical model describing how physiological traits, power output and anthropometric characteristics influence cycling speed. The effects of mass on overall performance are calculated by partitioning the independent effects of mass on energy required to overcome vertical displacement, air resistance (assuming increased mass is associated with increased anterior projected frontal area; FA) and rolling resistance. In a 26 km time trial with a 0% grade, an additional 1 kg BW is expected to cost a cyclist ~4.8 s (0.02%) most of which is due to a

predicted increase in FA (~3.6 s) with the remainder being due to an increase in rolling resistance. Mass has the greatest effect on energy required for vertical displacement with significant effects even over relatively mild grades. A 1 kg increase in BW will cost a cyclist 0.3% (9.6 s over 26 km) on a course with a 1% grade. Almost half (~4.3 s) of this is due to the increased energy required to overcome vertical displacement while the remainder is accounted for by the increase in FA (~3.6 s) and rolling resistance (~1.4 s). Races are often won by less than a second so, it is clear that small changes in non-functional mass can affect performance in time trials and solo breakaway attempts.

Small changes to body composition may influence the cyclists' ability to produce power in both absolute and relative (W/kg) terms as well as having subtle effects on rolling resistance and aerodynamic drag. To be able to monitor body composition and implement strategies to change it, accurate and reliable strategies for measuring body composition are required. The next section will describe the various body composition models and techniques used to measure them.

2.5 Measuring body composition

The earliest and most simplistic model of body composition is the two compartment model where the body is divided into fat mass (FM) and the remaining molecular components are collectively categorised as fat-free mass (FFM). Densitometry (see 2.5.1) and hydrometry (see 2.5.2) are the two underlying techniques upon which more complex models are based. Using densitometric models, FFM density is assumed to be 1.1 g/cm^3 and the proportion of FFM components (water, protein and mineral) are assumed to be stable. Given the difficulties associated with accurately measuring FM, FFM is frequently measured first and FM is assumed to make up the difference. If it is assumed that the water content of FFM is constant and FM is anhydrous, measuring total body water (TBW) can alternatively be used to determine FFM and FM. With the addition of TBW, it is possible to develop a three-compartment model which includes FFM, FM and TBW. The body can also be studied as a four compartment model by dividing FFM into protein and minerals (carbohydrates and vitamins are considered negligible and are excluded). While the two compartment model relies on the assumption of a constant FFM water content, the models that measure a higher number of compartments require fewer such assumptions. Ellis (2000) has suggested that models that reconstruct the body from the elemental level may well be more reliable as they reduce the need for assumptions of tissue density,

hydration, and/or structure. To clarify what specifically is measured in terms of body composition, Wang et al. (1995, 1992) presents the following five levels of body composition:

1. Elemental (oxygen, carbon, hydrogen, nitrogen, calcium)
2. Molecular (water, protein, lipids, bone mineral)
3. Cellular (cell mass, extracellular water, extracellular solids)
4. Tissue systems (skeletal muscle, adipose tissue, bone, blood)
5. Total body

There are a broad range of techniques used to measure body composition and all vary in their accuracy, practicality, the number of whole body tissue compartments they describe and expense. Accuracy of body composition measurement techniques depends in part on the objective of the analysis. To monitor the change in an athlete's body composition in the field, anthropometric measurements may be sufficient. However, when determining specific energy requirements of an individual based on body composition or to accurately quantify several compartments or regions of the body, more sophisticated techniques such as dual energy X-ray absorptiometry (DXA) or magnetic resonance imaging (MRI) may be required. While it is beyond the scope of this literature review to discuss these at length, Ellis (2000), Lee and Gallagher (2008) and Norgan (2005) provide comprehensive reviews of most techniques, while Fogelholm and van Marken Lichtenbelt (1997) provide a meta-analysis of the accuracy of techniques from 1985-1996. The various techniques are summarised below with a brief discussion about their potential use in athletic populations.

2.5.1 Densitometry

The underlying principle of densitometry is that if the body is made up of two components (FFM and FM) and the densities of these are known, whole body density is a function of the relative proportions of these two compartments. Density of the body was traditionally measured using underwater weighing given that density is equal to mass divided by volume; *Archimedes' principle*. This technique is highly repeatable, inexpensive and relatively safe. Underwater weighing has been used with athletes; however, it is not practical for routine use as it is time consuming and requires specialised equipment. More

recently, air displacement plethysmographic (ADP) apparatuses (BOD POD, COSMED Technology, Fridolfing, Germany) have been used, which do not require immersion. Air displacement plethysmography requires an estimate of BSA using the equation developed by Du Bois and Du Bois (1916), and is susceptible to some aberrant values possibly due to subject movement (Wells and Fuller, 2001). The validity of this method has been reviewed extensively (Biaggi et al., 1999; Dewit et al., 2000; Fields et al., 2002; Lockner et al., 2000; McCrory et al., 1995; Miyatake et al., 1999; Sardinha et al., 1998; Wells and Fuller, 2001). Ballard et al. (2004) have conducted one of the few experiments involving female athletes. Compared to DXA, there was no significant difference in the measurement of FM. Like DXA, the ADP units are expensive and not easily portable.

2.5.2 Hydrometry

If it assumed that FFM has constant water content, then the calculation of TBW can be used to determine FFM and the remaining mass is assumed to be FM. A stable isotopic tracer such as deuterium can be consumed and the concentration of this tracer in the blood, urine or saliva can be used to calculate TBW. The assumption of constant water content in FFM is subject to error due to ~2% biological variation in fluid shifts which can lead to erroneous estimates of FM (Jebb and Elia, 1993). With labelled water, the need for expert personnel, the expense of the stable isotopes and the 3 - 5 h required for equilibration in the body makes the technique best suited to well-funded research projects and less so as a tool for routine monitoring of athletes (Norgan, 2005).

2.5.3 Dual energy radiography (dual energy X-ray absorptiometry-DXA)

The principle underlying DXA is that when an X-ray is applied to one side of an object, the degree to which it is attenuated is proportional to the object's thickness, density and chemical composition (Ellis, 2000). This relatively new technique which was originally designed to measure bone density, now provides a three compartment model with precise measurements of bone mineral density (BMD), mineral-free FFM, and FM (Kiebzak et al., 2000). Van Der Ploeg et al. (2003) conducted a large scale study where FM calculated by DXA alone was compared to a four compartment model as the reference standard in 152 healthy adults. While the mean (\pm SD) difference for FM was only $1.8 \pm 2.0\%$, the intra-individual range was $-2.6 - 7.3\%$ and was consistently underestimated in the leaner individuals, which poses an issue for use with endurance athletes who are likely to be

lean. While a DXA machine is expensive and not portable, attractive features include its ease of use, short scan times (~10 min) and the very low dose of radiation (<5 millirem) required. Regional body composition measurements are also possible, although limited data are available on the validity of these measurements. Plank (2005) has reviewed the use and validity of DXA. Recent studies by Nana et al. (2012a, 2012b) provide recommendations for improving the reliability of DXA estimates of whole body composition in athletes. The recommendations are followed in our laboratory and include having the athlete present morning fasted in a euhydrated state which is confirmed by measuring their urine specific gravity (USG).

2.5.4 Computed tomography and magnetic resonance imaging

A computed tomography (CT) scan is similar to an X-ray but instead of taking a single one planar image, it takes a series of X-rays from multiple angles and reconstructs them to provide a three dimensional image of the body's organs or tissues. The greater number of X-rays increases the radiation exposure. Magnetic resonance imaging (MRI) works by applying a strong magnetic field which aligns the nuclear magnetic moment of unpaired protons and neutrons such as carbon and hydrogen. The MRI machine contains a coil that applies a radio frequency that alters this alignment. As the nuclei change alignment they transition through different energy states. The scanner then picks up the rotating magnetic field produced by the nuclei and creates a series of images of the cross-sectional area. Unlike CT, MRI gives no radiation exposure but the equipment and the measurement itself are very expensive; the subject must remain still for a relatively long time; and the apparatus can be noisy, which can be uncomfortable for subjects. Nonetheless, MRI has advanced to become the criterion measure for whole body and regional FM and FFM distribution and is the standard for calibrating field based measures (Ross et al., 2000).

Magnetic resonance imaging has been used successfully to measure thigh muscle volume in cyclists by Martin et al. (2000). In this study, thigh muscle volume was deemed representative of functional lean mass for sprint cycling performance. It was shown that thigh muscle volume explained 91% of the variance in peak power output in sprint cycling. In the same study, anthropometry (thigh girth and skinfolds) was used to estimate thigh lean mass which was also highly correlated ($R^2 = 0.76$) to peak power output. Maden-Wilkinson et al. (2013) compared MRI and DXA for estimating thigh lean mass and

although the two are strongly correlated ($R^2 = 0.88$), DXA underestimated the age related differences between young and old adults.

2.5.5 Anthropometry

Anthropometry is the measurement of BW, height, skinfolds, bone breadths and girths. Anthropometric techniques are popular field measures, particularly for use with athletic populations. They are quick to administer, easy and relatively non-invasive. While the precision of anthropometry is often regarded as being low, standardised training by the International Society for the Advancement of Kinanthropometry (ISAK) and the application of the standard ISAK protocols (Stewart et al., 2011) improves the intra- and inter-tester reliability of these data. Various population prediction equations can be applied to anthropometric data to estimate body density (BD) and from this percentage fat mass (Siri, 1961; Withers et al., 1987a, 1987b), although comparing raw results to reference data is often most useful. Most of the equations for BD cited here use equations to estimate percentage body fat (BF%) developed by either Siri (1961a) or Brožek et al. (1963). These make the following assumptions that may introduce error:

1. There is constant compressibility of the skin and subcutaneous fat
2. There are no differences in individual fat distribution patterns
3. There is a linear relationship between skinfold thickness and total body FM
4. The densities of FM and FFM are 0.900 and 1.100 g/cm^3 respectively
5. The proportion of the components of FFM (water, protein, bone mineral and non-bone mineral) do not vary between individuals
6. The densities of the components of FFM do not vary between individuals

Norton and Olds (1996) provide insight into the use and efficacy of anthropometric measurements. They postulate that the prediction equations developed by Withers et al. (1987a, 1987b) are most appropriate for use with elite male and female athletes respectively. The reported BD prediction errors (standard error of the estimate; SE) are 0.00533 and 0.00508 g/cm^3 , introducing errors for BF% of 2.4 and 2.4% for males and females respectively.

2.5.6 Bioimpedance analysis

The principle underlying bioimpedance analysis (BIA) is that the resistance of an object of homogenous material is proportional to its length and inversely proportional to its cross sectional area (CSA). In humans, BIA is performed by allowing a weak alternating current to flow from electrodes at the wrist to detecting electrodes at the ankle. The impedance to the current is directly proportional to the TBW. Once TBW is known, FFM and FM can be estimated. Single frequency BIA can estimate FFM but cannot determine differences between intracellular water (ICW) and extracellular water (ECW); however, this is possible with multi-frequency BIA can (Kyle et al., 2004).

Like anthropometry, population specific equations are necessary to estimate TBW precisely. Esco et al. (2011) recently used DXA as a criterion measure to compare BIA estimates of BF% and FFM in female college-age athletes. While measures using the two techniques were strongly correlated, BIA was seen to consistently underestimate FM and overestimate FFM. In contrast, Company and Ball (2010) found that BIA overestimated BF% in both power and endurance trained male athletes. Bioimpedance analysis requires further investigation into its validity in athletic populations but has a number of attractive features that should motivate researchers and manufacturers to improve the product. Bioimpedance analysis is easy to administer, inexpensive, non-invasive, does not require exposure to radiation and is highly portable. Potentially, this technology could be combined with DXA to measure TBW and FFM less TBW. This could be useful for athletes for whom changes in muscle mass may be of interest.

2.5.7 Body composition of female cyclists

While there are a number of contemporary articles describing the anthropometric and body composition characteristics of elite male cyclists (Campion et al., 2010; Fernández-García et al., 2000; Foley et al., 1989; Lee et al., 2002; Lucía et al., 1999; Menaspa et al., 2010; Mujika and Padilla, 2001; Penteadó et al., 2010; Rehrer et al., 2010; Smathers et al., 2009), only five papers were identified that address anthropometric characteristics of elite females cyclists (Burke, 1980; Huisveld et al., 1982; Martin et al., 2001; Pfeiffer et al., 1993; Wilber et al., 1997). The available data for females are summarised in Table 3. Martin et al. (2001) have published the most recent data detailing some distinguishing features of internationally competitive female cyclists. Female cyclists who were

internationally competitive National Team members were leaner (sum of seven skinfolds) and on average ~3 kg lighter than their less competitive team mates as established by their UCI ranking. This finding is different to data collected from male cyclists reported by Menaspa et al. (2010) in which there were no significant differences in BF% observed between low level professional riders (Professional Continental teams) and Pro-Tour professionals or between juniors who become professionals compared to those who did not. Relatively little research has focused on performance related differences in body composition, seasonal changes in body composition or the degree to which body composition can be manipulated. More recent normative data for the body composition of elite female cyclists are scarce, although Sherk et al. (2013) recently published a study monitoring bone density with DXA, which also included body fat (Mean \pm SD; 19.8 \pm 4.3%) and bone-free FFM (45.1 \pm 5.7 kg).

Table 3. Anthropometric and performance characteristics of American and Australian female cyclists

<i>Study</i>	<i>Participants</i>	<i>Age (y)</i>	<i>Height (cm)</i>	<i>Body Mass (kg)</i>	<i>FFM (kg)</i>	<i>BF%*</i>	<i>Skinfolds (mm)</i>	<i>MAP (W)</i>	<i>$\dot{V}O_2\max$ (ml/kg/min)</i>
(Wilber et al., 1997)	US National Road Team (n = 10)	26 ± 5	171 ± 5.0 (168 - 171)	60.4 ± 3.6	53.2 ± 3.0	11.9 ± 1.8 <i>Skinfolds</i> (Jackson and Pollock, 1978)	-	333 ± 21	63.8 ± 4.2
(Pfeiffer et al., 1993)	Participants in 1990 Idaho Tour (n = 16)	28 ± 3	-	61.0 ± 4.0	-	15.7 ± 2.0	-	-	64.2 ± 4.0
(Burke, 1980)	US National Road Team 1976 – 1980 (n = 7)	-	167.7 ± 10.7	61.3 ± 8.5	-	15.4 ± 4.7 <i>Hydrostatic weighing</i> (Brožek and Keys, 1951; Wilmore, 1969)	-	-	57.4 ± 6.6
(Martin et al., 2001)	Australian Women's Road Cycling Team	25.6 ± 4.6 (18 - 34)	(162-174)	59.9	-	13.8 ± 4.3 <i>Skinfolds</i> (Siri, 1961; Withers et al., 1987b)	58.9	291	-
(Martin et al., 2001)	Top 4 Australian Women's Team Road Cyclists	25.2 ± 3.1 (21 - 28)	-	57.0 (55.4 - 58.8)	-	9.3 (7-12) <i>Skinfolds</i>	42.2 (38 - 51)	308	63 - 70
(Huisveld et al., 1982)	Dutch elite Women including 1979 World Championship competitors (n = 11)	23.9 ± 4.4	170 ± 5.0	58.9 ± 4.9	46.7 ± 3.4	20.6 ± 4.1 <i>Hydrostatic weighing</i> (Brožek et al., 1963; Visser and Kowalski, 1972)	-	-	59.5 ± 8.0

Available results are presented as mean ± standard deviation (range). Fat free mass (FFM); body fat percentage (BF%); maximal aerobic power (MAP).

*The technique and reference to the equation used to calculate BF% are shown underneath the mean ± SD

2.6 Measuring energy intake

Energy intake is typically measured prospectively by recording details of food consumed by a subject at each meal throughout the day or retrospectively via food recalls with the period of investigation ranging from 1 - 7 d. The documented food (type and quantity) is then referenced to a database of food composition data (energy content, macronutrients and selected micronutrients). Accuracy of this approach generally relies heavily upon the subject's ability to keep accurate records of the type and quantity of food consumed. It is also important for the researcher/clinician to accurately match these descriptions to appropriate items in the food composition database which requires a good working knowledge of such databases. Johnson (2002) has reviewed the most common methods of measuring dietary intake. Traditional methods rely on information to be reported by the subject and include food records, food frequency questionnaires and 24 h recalls.

2.6.1 Food records

Food records require the subject to record the type and quantity of all foods consumed, typically for a period of 3 - 7 d. Broadly, the following are the two types of food records commonly used:

1. Estimated food records

- The quantity may simply be estimated by the subject or done so by using household measures or scales to quantify the food consumed.

2. Weighed food records

- All food consumed must be measured using standardised measuring cups and spoons and weighed using calibrated scales.

Food records have traditionally existed in paper form although, more recently, mobile applications (e.g. MyFitnessPal, Superfoods, Nutrino, My Diet Coach, WeightWatchers Mobile App etc.) for phones, tablets and other electronic devices have become popular. This technology gives the subject direct access to the database and gives the clinician or researcher almost immediate access to the uploaded data. Such applications are also starting to communicate with other portable devices that estimate physical activity, EE, sleep quality etc. The widely used online endurance athlete monitoring program

TrainingPeaks (Peakware LLC, Bolder, USA) incorporates a food record function which will then estimate EI and compare this to EE estimated from Global Positioning System (GPS), heart rate and power output data using proprietary algorithms. It is important with mobile applications to consider how culturally specific they are to the population studied as the databases frequently contain region specific foods.

2.6.2 Food frequency questionnaires

Food frequency questionnaires (FFQs) list specific foods and require subjects to indicate the frequency and quantity of the listed foods they consume. This can minimise the burden on the subject, particularly for longitudinal studies. Both long (100 items) and short (60 items) form FFQs exist and have been validated (Potischman et al., 1999) but they are not specifically designed to quantify EI. They more generally describe the nutrient intake for groups of individuals over prolonged periods (months). It is important for accuracy that these questionnaires be culturally specific to the foods and cooking practices of different ethnic groups (Johnson, 2002).

2.6.3 Twenty four hour recalls

The third type of dietary assessment is the 24 h recall. This technique involves a 20 - 30 min interview in person or over the phone where the subject describes everything consumed over the past 24 h. While this technique places fewer burdens on the subject compared to keeping a food record, it assumes that the reported intake for the past 24 h period is reflective of a typical day. As an extension of this method, the US Department of Agriculture developed the Multiple-Pass 24 h Recall (Conway et al., 2004) and more recently an automated version of this (Blanton et al., 2006; Moshfegh et al., 2008). This technique uses five passes to gather information about the subject's dietary intake. In the first pass the subject recalls everything eaten. In the second pass, the interviewer asks the subjects about specific foods that could have been forgotten. In the third pass the subject states when and where the foods were consumed. The fourth pass utilises standardised questions to determine portion size which are referenced to household measures like cups, spoons, bowls and glasses; geometric shapes such as circles, rectangles, and wedges; and finally actual measures of food mass. In the final pass, the interviewer makes a last attempt to record any other foods that may have been forgotten.

This process is still time consuming for both the subject and the researcher and still ultimately relies on the memory of the subject and a degree of subjectivity.

2.6.4 Validating dietary assessment tools

Measures of dietary intake have been assessed by comparing changes in body composition to *gold standard* measures of EE such as doubly labelled water (DLW; Poslusna et al. (2009). When body composition remains stable over a prolonged period of time but EI reflects an energy deficit, it is most likely that subjects are under-reporting EI. The *Goldberg Cut-Off* (Goldberg et al., 1991) calculates confidence intervals within which it could be reasonably expected that the EI reported is accurate, even in a sample with a proportionally high number of low reporters of EI. The *Goldberg Cut-off* is used as an index of EI relative to basal metabolic rate (EI/BMR) as it relates to the estimated EE. Black (2000a) has refined the equation and described its use, also improving its sensitivity by assigning activity levels (low, medium and high), for which different confidence intervals are applied (Black, 2000b).

2.6.5 Limitations of dietary assessments

Burke et al. (2001) have reviewed the inadequacies of self-report measures of dietary intake and identified the following issues pertaining to their use in athletic populations:

- when athletes self-report dietary intake they have been observed to change their eating behaviours during the period of observation
- athletes inaccurately report to give the appearance of a healthy diet
- athletes make errors in the quantification or type of food consumed

Poslusna et al. (2009) have reviewed the literature on misreporting in dietary assessments. They describe that under-reporting EI can result from both under-recording and under-eating. These two are sometimes differentiated by whether or not there is a change in BW during the assessment period or on the basis of findings from the validation techniques mentioned previously (DLW and the *Goldberg Cut-Off*). This meta-analysis included studies of healthy populations aged over 15 y. Energy intake was under-reported

by ~30% of subjects, while the magnitude by which EI was under-reported was approximately ~15%.

The studies identified by Poslusna et al. (2009) showed that women were more likely to under-report than men (Briefel et al., 1997; Hirvonen et al., 1997; de Vries et al., 1994). While these studies did not focus on female athletes, under-reporting has also been suggested to occur in Australian National Team female cyclists (Martin et al., 2002). Cyclists who wanted to reduce their fat mass consistently under-reported their dietary intake despite no changes in BW. The authors proposed that this behaviour was motivated by a desire to be perceived to be restricting intake due to social pressures and poor body image. More specifically, under-reporting appeared to be most prevalent in the female cyclists with the highest sum of seven skinfolds. This observation is supported by Edwards et al. (1993) who found that female runners with higher BW, self-report poorer body image scores and lower EI, resulting in significant energy imbalances despite no change in BW. Such problems may be minimised but probably not eliminated by emphasising the purpose of the study to athletes, explaining how the collected data will be analysed and by educating them on how to accurately measure and document their intake. Of course, it is also possible that athletes with higher body fat levels and an apparent mismatch between EI and EE are exhibiting the outcome of reduced metabolic rate following periods of low EA induced by past attempts to reduce body weight (Vanheest et al., 2014). Therefore, the extent to which current EI is under-reported, may be overestimated since it is not being compared with actual suppressed EE.

2.6.6 The nutritional practices of cyclists

No known studies have directly addressed whether female cyclists are concerned about their weight and leanness. However, experience at the Australian Institute of Sport suggests that coaches and athletes are motivated to develop physiques that have very little mass in the upper body, and as little total FM as possible. As reviewed earlier, there is a strong basis for this desire; however, there are significant challenges that come with achieving this in a healthy, tolerable way that meets performance goals. Elite cyclists travel extensively, challenging their ability to sustain a consistent nutritious dietary routine that supports training while at the same time changing or maintaining body composition. Travel adds to fatigue and introduces unfamiliar foreign foods which can further contribute to inconsistent eating patterns. For most female cyclists, there is little financial reward

even at the international professional level. Salaries rarely exceed €25,000 except in exceptional circumstances and in many cases, athletes are only provided with basic living expenses and equipment. Therefore, training must be juggled around work and study commitments as well as appointments typical of sporting life (medical, physiotherapy, massage, etc.).

Cycling culture is unique and strong. While sports like the football codes are followed by people of all ages, few actually play the sport. In contrast, competitive cycling enjoys a vast following as well as participation from the recreational to professional level from almost all ages. *USA Cycling* boasts ~75,000 members comprising 2,812 clubs (*USA Cycling Annual Report, 2013*) while *Cycling Australia* has ~25,000 members in over 200 clubs (*Cycling Australia: Annual Report, 2013*). Ingrained in this cultural identity is communication regarding advancements in bicycle technology, race strategies, etiquette, fashion, training techniques, training methodology and nutrition. Much of this is communicated through word of mouth as cyclists congregate for local bunch rides or club races but equally so through the written word; ideas and opinions are spread widely and quickly via the internet, magazines and television coverage of the sport. Few opinions are based on empirical data derived through scientific studies. Some teams may have well qualified staff such as medical doctors, physiotherapists, dietitians and physiologists, who provide expert support based on credible evidence. Team *soigneurs* play an integral role by preparing food and drink (pre-race, race, and post-race), manning feed stations, giving massages and transporting the riders. *Soigneurs* have a great deal of direct contact with athletes so, they become another common source of nutritional advice.

Cyclists are quick to adopt practices that promise performance benefits, particularly if the practice is used by successful competitors. While this drive for a competitive edge fuels innovation in cycling, it also encourages arbitrary use of supplements and dietary interventions that not only lack scientific merit but may risk harm to the athlete. By the same token, much of what athletes do in practice has a strong rationale and studies conducted by sports scientists sometimes just confirm what athletes already believe to be true. Burke (2007) gives the example of the consumption of de-carbonated cola by cyclists consumed late in the race for its glucose and caffeine content; a practice that initially was not well supported by research but has more recently been confirmed as beneficial.

The nutritional practices of cyclists have been reviewed by Burke (2001). Three studies have observed the nutritional practices specifically of elite female cyclists, although these are quite dated (van Erp-Baart et al., 1989a, 1989b; Keith et al., 1989). One study provides insight into how much energy female cyclists expend and consume while none have explored the efficacy of specific strategies that manipulate body composition.

2.6.6.1 Training diets

Broadly, there are two types of diets cyclists engage in; training diets and competition diets. Training diets can be highly variable depending of the volume and intensity of training and season phase. Training diets are geared towards providing adequate nutrition to meet the energy requirements of the training load, facilitate and possibly enhance training adaptations, maintain or alter body composition, accelerate recovery, maintain health, and provide opportunity to practice race nutrition strategies. Martin et al. (2002) found a strong correlation between the amount of energy expended through training and the dietary EI of cyclists ($r = 0.78$). Participants were monitored during high and low training load days. Some athletes in this study targeted a negative energy balance (EB) on recovery days to reduce BW. Keith et al. (1989) reported that female cyclists consumed only 85% of their recommended daily allowance (RDA) of energy, and consumed insufficient carbohydrate (4.4 g/kg/day) as well as sub-optimal amounts of essential micronutrients. Given that male cyclists have been shown to be at risk of subclinical eating disorders (Riebl et al., 2007), it is quite possible that poor nutrition and ill-informed weight loss strategies exist within women's cycling. The typical diet of endurance cyclists should be characterised by high carbohydrate (CHO) availability for key training sessions and racing. For males, CHO consumption is reported to be in the range of 8 to 12 g/kg/day for such periods, as this is sufficient to restore muscle and liver glycogen stores during high volume training (Burke, 2001). The EI and macronutrient composition of diets adopted by female cyclists during competition and training (Martin et al., 2002) are shown in Table 4.

Some athletes undergo periods of high volume training and very low EI to rapidly reduce BW. Vogt et al. (2005) observed 11 professional male cyclists during a six day pre-season camp specifically designed to reduce BW. Energy intake was only 67% of their estimated EE and BW was reduced by an average 730 g. Similar strategies may be adopted by female cyclists. Details of more extreme weight loss strategies allegedly used by

professional cyclists have been published in a popular cycling magazine (Matheny, 1997) and include:

- Complete a long (3 - 4 h) ride in a fasted state to promote fat oxidation while in a CHO depleted state;
- Eat a standard breakfast, complete a 5 -7 h ride consuming only a CHO drink, eat a low CHO post-ride meal and fast until the following morning to promote fat utilisation during sleep;
- Suppress appetite with *diet drugs*; and
- Induce a negative energy balance throughout a stage race.

2.6.6.2 Competition diets

Cyclists have multiple opportunities to receive nutrition throughout a bike race. In addition to carrying two bottles of water and/or carbohydrate-electrolyte drink (e.g. *Powerade*, *Gatorade*, etc.), the pockets on their jerseys allow the athlete to carry carbohydrate dense foods in the form of bars or gels and sometimes prepared food such as small tarts, sandwiches etc. In addition to these sources of energy, cyclists can return to their team vehicle which travels in the *race convoy* behind the *peloton* to receive food and drink, however, this comes at a high energy cost and places the rider in an unfavourable position; behind the *peloton*. In most races, teams will have one or two riders who will be supported by the rest of the team so that these protected riders never have to return to the team vehicle. Union Cycliste Internationale rules stipulate that such feeding cannot take place during the last 20km of a race. The other opportunity riders have to receive food is from *feed zones*. These are designated stretches of road along which the team *soignier* stands and hands out bottles and small bags (*feedbags*) containing food to the passing riders. Competition diets are influenced by the type of race and include specific strategies for before, during and after races. Cyclists will often compete in single-day events but competition formats also include multi-day stage races like the *Giro d'Italia Femminile Internazionale*, where cyclists accumulate approximately 800 km per week. Unlike some sports that compete relatively infrequently or have short seasons, elite female cyclists can have anywhere from 40 to over 70 race days in a season, stretching from early January and through until September, at the end of which the World Championships take place. Therefore, race specific nutrition occupies a significant component of the regular dietary

routine. During competition, Australian National team female cyclists have been reported to consume an average of 14.87 MJ/d or 0.247 MJ/kg/d (Martin et al., 2002). The macronutrient content of this diet is shown in Table 4.

2.6.7 Summary of energy intake

The most accurate method for measuring EI is to use weighed food diaries, although even this technique can result in significant alteration of usual food intake and under-reporting. Educating subjects to weigh food accurately and describe portion size may help but psychological and emotional factors may also contribute to under-reporting. Tools such as smart phone applications may be the future of EI quantification.

Cyclists have higher EI requirements than many other athletes due to the high levels of EE. Elite female cyclists consume ~14 - 15 MJ/d of which ~65 % is CHO. They engage in diets which are influenced by training and racing as well as body composition goals and logistical constraints. Some dietary practices can be quite extreme and may pose a risk to health. No known studies have compared the efficacy of different dietary and training strategies aimed at manipulating body composition in elite female cyclists. Future research should focus on strategies that incorporate both diet and training with consideration for health as well as performance.

Table 4. Energy intake and macronutrient composition of diets in Australian National Team female cyclists during training and competition

	<i>Energy</i>		<i>CHO</i>			<i>Protein</i>			<i>Fat</i>		
	<i>MJ</i>	<i>MJ/kg</i>	<i>g</i>	<i>g/kg</i>	<i>%EI</i>	<i>g</i>	<i>g/kg</i>	<i>%EI</i>	<i>g</i>	<i>g/kg</i>	<i>%EI</i>
<i>Heavy training</i>	14.11	210	536	9.1	63	158	2.7	20	65	1.1	17
<i>Recovery training</i>	11.98	179	448	7.6	63	130	2.2	19	59	1.0	18
<i>General racing</i>	14.87	222	588	10	67	136	2.3	16	67	1.1	17

Adapted from Martin et al. (2002) with permission.
Carbohydrate (CHO); mega joules (MJ); energy intake (EI).

2.7 Measuring energy expenditure

Quantification of both EI and EE is required to calculate EB. The following are the primary components of total daily EE that should be considered in athletic populations:

- Basal metabolic rate (BMR)
- Thermic effect of food (TEF)
- Activity thermogenesis
 - Exercise energy expenditure
 - Non-exercise activity energy expenditure

Although measuring EE is typically attempted under laboratory conditions, there are also a number of field based techniques that exist. The three broad approaches used to measure EE include indirect calorimetry, direct calorimetry, and field based methods. The strengths and weaknesses of these techniques have been reviewed by Levine (2005).

2.7.1 Indirect calorimetry

Indirect calorimetry is a technique that relies on the collection of expired gases from which the volume and concentration of oxygen (O₂) and carbon dioxide (CO₂) are measured. Heat production and thus EE are then calculated using equations that are based on the stoichiometric relationship of the products and reactants of oxidative metabolism. Indirect calorimetry can be performed using closed or open circuit systems. Closed collection systems require subjects to inspire air from a pre-filled system that contains oxygen. The expired gas then passes back into this system via a canister containing soda lime which removes the expired carbon dioxide. The system collecting expired air is a rigid structure such as a Tissot Gasometer or a flexible collection system such as a Douglas bag. Quantification of the volume of air and the fractional concentration of oxygen in the inspired and expired air, allows for the calculation of oxygen consumed. The volume of oxygen consumed ($\dot{V}O_2$) and the respiratory exchange ratio ($\dot{V}CO_2 / \dot{V}O_2$) can be used to estimate EE. While accurate, such systems are not practical for measuring EE during physical activity in the field. Open circuit systems allow the subject to inspire ambient air.

Three types of open circuit systems are available:

- portable spirometry
- bag collection technique
- computerised systems

Portable systems allow measurements to be made in the field as the subject performs an activity while wearing the apparatus as a backpack. A gas analyser takes a sample of the ambient air to determine the concentration of the carbon dioxide and oxygen inspired. The exhaled air also passes through a gas analyser which determines the concentration of these gases. Computerised systems have flow meters that continuously measure the air volume inspired as well as oxygen and carbon dioxide analysers to record the fractional concentration of the expired gas. The bag technique collects the expired air in a balloon or Douglas bag which can be emptied periodically (every ~30 s) to determine the volume and concentration of gases using gas analysers.

The three methods of indirect calorimetry discussed can be used to assess the oxygen consumption during a bout of exercise. In contrast, whole-room indirect calorimetry can be used to measure $\dot{V}O_2$ and therefore EE for extended periods, even several days. Subjects are placed in an air sealed room of known volume and gas concentration. Gas analysers measure the changes in gas concentration to determine oxygen consumption and carbon dioxide production. The chamber is flushed periodically with *fresh air* to prolong the duration of observation. These systems allow EE to be measured while the subject performs various tasks of daily living as well as sleeping. The primary limitation of this approach is that the subject is confined to the chamber.

Indirect calorimetry allows for the measurement of EE before, during and after bouts of exercise, during rest and even during sleep. With the exception of whole-room indirect calorimetry, a mouth-piece or a hood must be worn which can be uncomfortable and limit the duration of data collection. Indirect calorimetry is widely used and highly accurate when performed by trained technicians and with calibrated equipment. Aside from the use of portable systems such as the Metamax 3B (Cortex, Leipzig, Germany) or the Cosmed K4 b² (Cosmed, Rome, Italy), which have been previously validated (Leprêtre et al., 2012; Macfarlane and Wong, 2012), indirect calorimetry is used infrequently in the field due to the restrictions the equipment place on the athlete's ability to perform physical activity.

2.7.2 Direct calorimetry

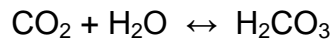
Direct calorimeters measure the heat loss from living animals, including humans, which is used to estimate EE. Heat is lost via radiation to surrounding surfaces; convection to the surrounding air; conduction to objects the subject is in contact with; and via evaporative pathways. Spinnler et al. (1973) described the isothermal calorimetry chamber which is lined with a layer of insulating material. The inner aspect of the insulation is kept at the same temperature as the inside of the room while the outside aspect is kept at the same temperature as the chamber wall. The wall is kept at a constant temperature by a circulating fluid. As subjects inside the chamber dissipate metabolic heat, a proportional temperature gradient is created across the insulating layer and EE can be calculated. Adiabatic or heat sink calorimeters are different in that they allow no heat exchange across surfaces of the chamber. Instead, heat generated by the subject is removed by a liquid-cooled heat exchanger at a rate that prevents a thermal gradient between the inner and outer chamber walls. Finally, convection calorimeter chambers are insulated to prevent heat loss through the surfaces and the measurements are based on the mean temperature rise of air ventilating the chamber at a known rate (Reardon et al., 2006; Snellen, 2000; Snellen et al., 1983; Tschegg et al., 1979). While whole room direct calorimetry allows for very accurate measures of EE for prolonged periods, these systems are extremely expensive to build and operate; have slow response rates (3 – 30 min) compared to indirect calorimetry; and like whole-room indirect calorimeters, they confine subjects to the chamber, which may change their typical behaviours. Direct calorimeters allow for the quantification of each of the components of total daily EE but their availability, cost and spatial restriction are limiting factors, particularly with athletic populations.

2.7.3 Doubly labelled water

The doubly labelled water (DLW) technique was first described by Schoeller and van Santen (1982) and is considered a *gold standard* technique for measuring EE in free living subjects. As described by Ainslie et al. (2003), DLW can be used to measure the total daily EE of free living subjects for durations of 4 - 20 d by measuring the rate of CO₂ production. Subjects consume an oral dose of non-radioactive labelled water containing known amounts of isotopes for hydrogen (²H; deuterium) and oxygen (¹⁸O). The ²H and ¹⁸O equilibrates with unlabelled hydrogen and oxygen in the body water within a matter of hours. The principle underlying DLW is based on the dynamic equilibrium of water, carbon

dioxide and carbonic acid (H_2CO_3), which is shown in Equation 2. Based on this relationship, labelled ^{18}O will be distributed not only within body water but also circulating H_2CO_3 and CO_2 which will be expired.

Equation 2



Water and CO_2 are produced along the metabolic pathways. The ^{18}O is liberated from the body in the form of water as well as C^{18}O_2 from respiration, whereas deuterium is only liberated as water. Therefore, ^{18}O leaves the body more rapidly than does deuterium. To determine the rate of CO_2 production, the difference in the elimination rates of ^{18}O and ^2H is determined. Changes in deuterium and ^{18}O concentrations in the body water are measured by sampling urine, saliva and blood and using spectroscopy. The ^{18}O not accounted for through body water losses is assumed to have been lost from the body via respiration as C^{18}O_2 . As a minimum, measurements are taken at the start and end of the trial, although more frequent measurements can improve accuracy and illustrate the rate of total daily EE on specific days. With CO_2 production known, O_2 consumption and thus EE can be calculated. In order to do this accurately, an appropriate estimate of respiratory quotient (RQ) is needed. Black et al. (1986) have calculated the food quotients of a number of different broadly classified diets and have suggested that omnivorous adults have an RQ of 0.845 ± 0.013 . Alternatively, the food quotient can be calculated for each individual based on dietary records. Either method assumes that the amount of macronutrient oxidized is equal to the amount consumed which may only hold true when the subject is in EB. De Jonge et al. (2007) have observed energy restricted subjects using DLW and indirect calorimetry simultaneously while also consuming standardised meals. Meals with an average food quotient of 0.882 were consumed but the mean 24 h RQ was 0.84 ± 0.03 resulting in a higher (60 kcal) estimated total daily EE with the latter. Conversely, Schoeller and van Santen (1982) observed subjects under similar conditions, however, they were in EB. In this study the food quotient was 0.845 while the mean 24 h RQ ranged from 0.848 to 0.889 and total daily EE was 66 kcal greater using the food quotient. These examples illustrate that the RQ used must reflect the diet and EB of the individuals observed.

While DLW is somewhat expensive, the fact it can be used by subjects under free living conditions presents a major advantage for use with athletic populations. Doubly labelled water is often used to validate other indirect methods for measuring total daily EE, however, outside of the context of research, it is not suitable for estimating EE in athletes. There is a growing market for commercially available devices that are portable and wearable that may be useful with athletic populations.

2.7.4 Power meters

Exercise activity thermogenesis or exercise EE can form the greatest component of total daily EE for endurance cyclists. As mentioned earlier, many cyclists use commercially available power meters as a training tool in order to measure mechanical work performed. For a given training session, the power meter will allow the quantification of average power (W) and duration. Joules of work can then be calculated (i.e. 1 W = 1 J/s). Once the total mechanical work is known, assumptions must be made about the efficiency of the cyclist so that metabolic EE can be estimated. Efficiency can be quantified and described differently using the following calculations (Ettema and Lorås, 2009):

- Net efficiency = power / (metabolic rate – resting metabolic rate)
- Work efficiency = power / (metabolic rate – unloaded metabolic rate)
- Delta efficiency = change in power / change in metabolic rate
- Gross efficiency = mechanical power / metabolic rate

Efficiency can be viewed as a performance parameter, but in the context of this thesis, it is used only to estimate EE. From this perspective, gross efficiency (GE) is the preferred metric as it is affected by changes in homeostatic EE, rather than only work, and therefore best reflects total EE during the exercise period.

In some cases, cyclists assume a GE of 25% (Vogt et al., 2005). Most power meters display work expended in kilojoules (kJ). Given that there is ~4 kJ for every kilocalorie (kcal), some cyclists take advantage of this near 4:1 relationship and assume that 1 kJ of mechanical EE performed is equal to 1 kcal of metabolic EE. A source of error comes from the fact that 1 kcal is in fact 4.186 kJ, which can make a significant difference when

quantifying the EE of rides lasting several hours. Further, the estimation of a given efficiency for all types of cycling and for all cyclists may be too simplistic to accurately estimate EE during cycling.

Measures of cycling efficiency are complicated by a number of factors. The two factors most commonly discussed are work rate and cadence (Chavarren and Calbet, 1999; Ettema and Lorås, 2009; Gaesser and Brooks, 1975; Gonzalez and Hull, 1989; Lucia et al., 2004; Marsh et al., 2000; Moseley and Jeukendrup, 2001). Ettema and Lorås (2009) have reviewed the cycling efficiency literature and concluded that the relationship between GE and external power is generally curvilinear. This is likely due to the diminishing contribution of baseline metabolic rate to total EE as work rate increases. This review reports that 91% of the variance in GE is explained by work rate, with the remaining variance explained by cadence. Gross efficiency is most typically observed to decline slightly as cadence increases across a range of ~60 – 120 rpm (Ettema and Lorås, 2009). In contrast, Lucia et al. (2004) have reported the opposite in professional male cyclists riding at high power outputs (366 ± 37 W). In this study, GE at a cadence of 100 rpm was significantly higher ($24.2 \pm 2.0\%$) compared to at 60 rpm ($22.4 \pm 1.7\%$). Possible explanations include reduced type II motor unit recruitment and improved effectiveness of the skeletal-muscle pump in facilitating venous return during the higher cadence trials. It is noteworthy that elite cyclists tend to use cadences that are closer to 100 rpm and, in this study, the rate of perceived exertion (RPE) and blood lactate measures were lower in the high cadence trials also.

Assumptions of cycling efficiency in the field may not be entirely valid, as a result of non-steady state cycling, which characterises most forms of road cycling. Sahlin et al. (2005) have shown that GE is reduced when cycling at a moderate intensity following three high intensity ($110\% \dot{V}O_{2max}$) efforts. This suggests that efficiency is attenuated following supra-maximal exercise. Likewise, Passfield and Doust (2000) have shown that GE decreases following 60 min of moderate intensity cycling. To further complicate matters, other studies provide evidence that bike position (Browning et al., 1992; Gonzalez and Hull, 1989), crank design (Santalla et al., 2002), pedalling technique (Korff et al., 2007), diet (Cole et al., 2014; Poole and Henson, 1988), body mass (Berry et al., 1993), age (Sacchetti et al., 2010), genetics (Cortright et al., 1999), training status (Hopker et al., 2009, 2010a, 2010b, 2007), overtraining (Bahr et al., 1991), fibre type distribution (Coyle et al., 1992), and sex specific variations in lean leg volume (Hopker et al., 2010a) can affect

cycling efficiency. In short, there may be considerable intra- and inter-individual variability in estimates of efficiency and thus EE. While convenient, the assumption that GE is simply 25% for all individuals and all forms of cycling is likely erroneous. Based on the existing literature, the majority of studies have reported values substantially below 25%, across a range of pedalling frequencies and power outputs (Ettema and Lorås, 2009) suggesting that this method would likely underestimate EE.

While differences in efficiency may seem subtle, estimates of exercise EE may be highly variable depending on the efficiency value used. If a cyclist completes a 4 h ride with an average power output of 150 W, the total external work performed is equal to 2160 kJ. Using an efficiency of 20% would result in an estimated total metabolic EE of 10.8 MJ while at 25% it would be 8.64 MJ. Such differences can lead to vastly different estimates of EE, particularly when used over extended periods of high volume training as would be expected with elite cyclists. While efficiency is influenced by a number of factors, one which has not been explored thoroughly is the influence of more variable intensity cycling which better represents the stochastic nature of road cycling. While a power meter may read an average power of 150 W, training and racing are characterised by great oscillations in power output. It is unknown what effect stochastic cycling has on efficiency and thus the accuracy of estimating EE using power meters during such exercise.

2.7.5 Heart rate monitors

The use of heart rate (HR) monitoring to estimate EE is based on the principle that there is a linear relationship between HR and $\dot{V}O_2$ during sub-maximal exercise. Heart rate monitors therefore present an attractive approach to estimating EE as they are inexpensive and easy for the athlete to use. A HR - $\dot{V}O_2$ regression must be established for each individual to account for differences in fitness (Christensen et al., 1983) as well as differences in resting and maximum HR. Once the HR - $\dot{V}O_2$ relationship is established, HR can be used to estimate $\dot{V}O_2$ and thus EE by using stoichiometric equations. The preferred method for estimating EE in this way is the FLEX HR method which was first described by Spurr et al. (1988). This method establishes the individual HR - $\dot{V}O_2$ regression for lying down, sitting, standing and exercising at various intensities. Resting metabolic rate (RMR) can also be measured for each individual. A threshold or FLEX HR is used to differentiate between EE associated with rest and physical activity. This threshold is identified as the highest HR measured at rest or during sedentary activity and

the lowest HR associated with very light physical activity. The EE associated with RMR is applied to every minute that the HR is below the FLEX HR. For every minute that HR is above the FLEX HR, the HR - $\dot{V}O_2$ regression is used to calculate EE. The downside of this method is that it is time consuming for the physiologist and the athlete. It also relies on the assumption that the HR - $\dot{V}O_2$ relationship that occurs in the field will be the same as that observed in the laboratory (Livingstone, 1997). A major limitation with all HR based estimates of EE is that HR may change as a result of many factors other than physical activity and the degree to which these are related to metabolic rate may differ. Heart rate is influenced by caffeine (Green et al., 1996); environmental temperature and hydration status (Montain and Coyle, 1992); altitude and the athlete's physical state (Jeukendrup and VanDiemen, 1998); and even bike position (Gnehm et al., 1997). Heart rate also fails to provide a high resolution of EE as it has a delayed response to instantaneous changes in physical activity. These reasons may explain why errors in 24 h estimates of EE made using HR can be as great as ~20% compared to estimates made using DLW (Davidson et al., 1997) and whole room calorimetry (Spurr et al., 1988)

2.7.6 Motion sensors

Motion sensors have also been used as a field based approach to measuring EE. Motion sensors (accelerometers) measure the movement or displacement of a limb or the trunk depending on their placement. These devices range from very cheap pedometers which simply count stride rate, through to multi-axial accelerometers that measure total body displacement and allow data to be downloaded and analysed. Pedometers allow easy measurement of non-exercise activity thermogenesis such as ambulation, but since they do not measure stride length or total body displacement they have reduced ability to accurately quantify EE. Accelerometers function on the premise that acceleration is proportional to muscular forces and therefore EE. By measuring the amount and intensity of movement, estimates of EE over periods of hours and even days can be calculated. Errors with this technique are associated with the inability of such devices to include load carriage and changes in gradient. This results in large errors particularly for high intensity exercise. Uniaxial accelerometers tend to measure accelerations in the vertical plane only. Triaxial accelerometers measure accelerations in the anteroposterior, mediolateral, and vertical planes, which results in greater accuracy across a broader range of physical activities. Of course, accelerometers can only be expected to estimate EE associated with physical activity. Because of this, validation studies using DLW sometimes measure BMR

or sleeping metabolic rate (SMR) using indirect calorimetry. Energy expenditure resulting from physical activity is then estimated to be 90% of total daily EE less BMR (Plasqui and Westerterp, 2007). This assumes that the thermic effect of food is 10% of total daily EE. With BMR and physical activity EE known, comparisons can be made to *gold standard* measures of total daily EE e.g. DLW.

A meta-analysis by Plasqui and Westerterp (2007) has shown that uniaxial accelerometers such as the Actigraph (Health One Technology, Fort Walton Beach, FL, USA) only have a low to modest correlation with DLW estimates of total daily EE. The Tracmor triaxial accelerometer has shown better correlations ($r = 0.63 - 0.91$). As is pointed out in this analysis, the studies that assessed these devices rarely evaluate whether the activity counts added significantly to the regression equations which also include subject characteristics such as age, gender, height and BW. As an exception, a study by Plasqui et al. (2005) found that age, BW and height already explained 64% of the variation in total daily EE, while the Tracmor accelerometer explained a further 19%. The regression equation used, therefore explained 83% of the variation in total daily EE but had a standard error of the estimate (SE) of 1.00 MJ/d.

Motion sensors are typically small, light and easy to wear, placing little burden on the subject. Their pitfall, like HR monitors, is their lack of accuracy. Pedometers and uniaxial accelerometers may serve to quantify group activity levels, whereas triaxial accelerometers are better for quantifying EE at an individual level. In a population of cyclists, they could be used to determine non-exercise activity EE, but would be inappropriate for use on the bicycle as they only respond to accelerations and not the constant motion associated with cycling (Matthew, 2005).

Multi-sensor arm bands are a recent innovation that attempt to overcome the limitations imposed by other methods of motion analysis, by incorporating physiological responses to activity as well as motion. Berntsen et al. (2010) have compared a number of activity monitors to indirect calorimetry during a series of moderate and vigorous physical activities of daily living. Monitors included the ActiGraph 7164 uniaxial accelerometer; the ActiReg (PreMed AS, Oslo, Norway), which measures body position and body motion using a sensor placed on the chest and one of the thigh; the ikcal (Teltronic AG, Biberist, Switzerland), which measures HR and acceleration in the horizontal and vertical planes; and the SenseWear Pro2 Armband (BodyMedia, Pittsburgh, Pennsylvania, USA), which

measures motion using a triaxial accelerometer, galvanic skin response due to sweat using an electrodermograph, and detects skin temperature and heat flux using a thermistor. The SenseWear and the Actigraph overestimated time spent in moderate and vigorous physical activity by 2.9% and 2.5% respectively. The iKcal and ActiReg underestimated this by 11.6% and 98.7% respectively. All the monitors underestimated total EE for a 120 min bout of activity, although the SenseWear provided the best estimate. Koehler et al. (2011) compared EE measured using the SenseWear Pro2 Armband to indirect calorimetry in endurance athletes and found significant individual errors (1368 to 1238 kcal/d), concluding that the SenseWear Pro2 Armband was inappropriate for use during high intensity cycling and running. The SenseWear band has been validated in free living adults (Berntsen et al., 2010; Fruin and Rankin, 2004; St-Onge et al., 2007) and provides accurate measures of non-exercise components of total daily EE. SenseWear Pro3 now includes a triaxial accelerator while the Pro2 was only biaxial and Bodymedia continue to refine the software algorithms used to calculate EE. It is our experience that the SenseWear armband greatly underestimates EE during both stationary and road cycling. In its current form, the SenseWear armband could potentially be used with tools that are more established for estimating exercise EE, such as power meters and HR monitors. With cyclists, this may provide a more comprehensive estimate of total daily EE. Although conceptually appealing, research on this topic remains to be conducted.

2.7.7 Energy expenditure of female cyclists

Understanding the EE of endurance cyclists is desirable when attempting to manipulate or maintain body composition. Similarly, understanding EE during periods of intense training or racing can be important as it has been shown that even short-term energy deficits can result in symptoms of overreaching (Halson et al., 2004) while longer term energy deficits can result in reproductive dysfunction and poor bone health (Burke, In Press; Loucks et al., 2011; Manore et al., 2007; Mountjoy et al., 2014; Nattiv et al., 2007; De Souza et al., 2014). An obvious characteristic of elite endurance athletes is the large volume of physical activity engaged in daily, which dramatically increases total daily EE. Sometimes this is quantified as the average physical activity level (PAL), which is defined as the ratio of total daily EE to BMR. In the general population, this is seen to be ~1.6 - 1.7 whereas endurance athletes can reach PAL values of ~4.0 - 5.0. Westerterp (2003) have shown that in some athletes, 70% of total daily EE was made up of exercise EE and so accurately

quantifying this becomes critically important when measuring total daily EE for the purpose of manipulating body composition.

Using whole-room indirect calorimetry, Horton et al. (1994) measured the contribution of RMR, the thermic effect of food, non-exercise activity EE and exercise EE towards total daily EE in a group of sedentary females and a group of recreational female cyclists. Figure 1a and 1b illustrates the relative contribution of these components towards total daily EE for each group. The trained cyclists only rode enough to expend 3.17 ± 0.79 MJ of energy (~90 - 120 min). We know that elite female cyclists regularly perform 3 - 5 h training rides as well as various forms of strength and cross training. Martin et al. (2002) reported the average daily exercise EE in elite female cyclists during days of training and racing as 7.65 ± 1.20 MJ and 9.46 ± 0.79 MJ respectively. If exercise EE increases to 9.46 MJ and the absolute RMR, thermic effect of food and non-exercise activity EE remain constant (same as the recreational cyclists), then the relative contribution of exercise EE becomes the greatest component of total daily EE (Figure 1c). Accurately estimating exercise EE is therefore important in this population. To further emphasise this, a 5% error at these levels of physical activity would result in a ~ 0.5 MJ error in total daily EE.

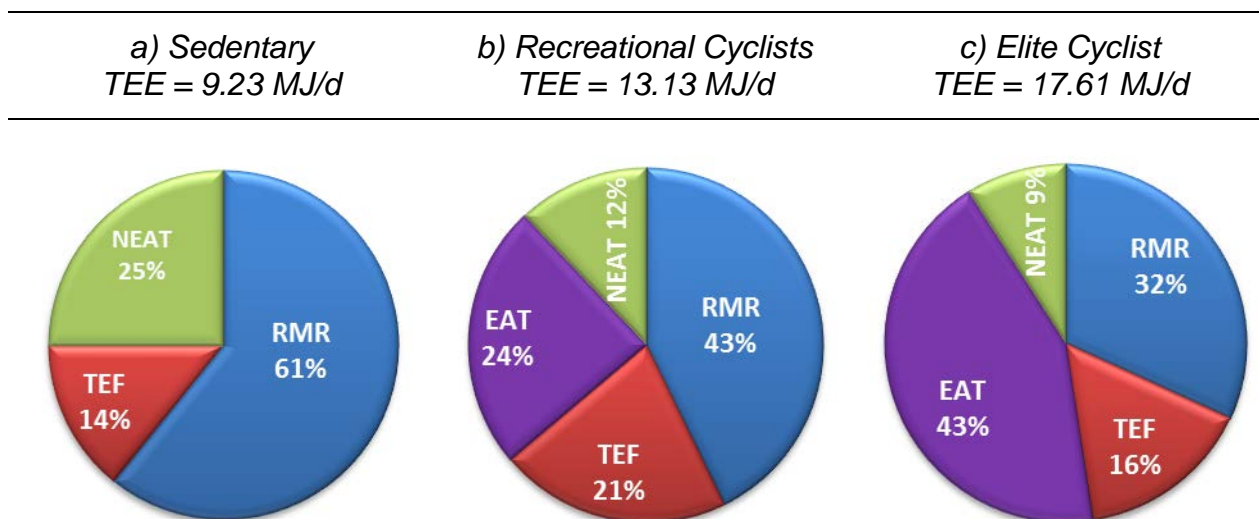


Figure 1. Relative contribution of components of total energy expenditure in sedentary females as well as recreational and elite female cyclists. Components include the resting metabolic rate (RMR); thermic effect of food (TEF); non-exercise activity thermogenesis (NEAT); and exercise activity thermogenesis or the energy expenditure associated with exercise (EAT). Values adapted from (Horton et al., 1994) and (Martin et al., 2002).

The EE and EI of elite male cyclists have been studied during a *Tour de France* simulation using whole room calorimetry (Brouns et al., 1989a, 1989b). Similarly, DLW has been used to estimate EE of cyclists in the actual *Tour de France* (Westerterp et al., 1986) and in the *Tour of Southland* (Rehrer et al., 2010). While racing EB is important for performance, particularly during stage races lasting several days or weeks, it is typical for body composition to be manipulated during periods of training. Similar to the protocol of Martin et al. (2002) involving female cyclists, Vogt et al. (2005) used SRM power meter data (assumed efficiency of 25%) and food diaries to estimate EE and EI during training. More research is required to determine the best practice for estimating EE using valid field-based techniques which can be routinely used during both training and competition.

2.7.8 Summary of methods for quantifying energy expenditure in female cyclists

Female cyclists expend between ~15 - 20 MJ/d depending on the volume and intensity of training and racing. While calorimetry presents an accurate method for quantifying exercise EE, even portable systems cannot be used in the daily training environment and certainly not during competition. Given the wide spread use of power meters, these present as convenient tools for quantifying cycling EE in the field. No studies have validated the use of power meters for this purpose during sub-maximal stochastic cycling representative of road cycling. Determining valid and reliable methods for using power data to estimate exercise EE and combining this with non-exercise EE estimates from devices such as the SenseWear Armband, may be a practical and accurate solution for quantifying total daily EE for road cyclists. The summation of EE measured by these combined techniques could be validated against *gold standard* techniques such as doubly labelled water.

2.8 Physiological responses to changes in energy availability

Energy balance is calculated as the difference between total daily EE and dietary EI (see Equation 3a). In contrast to EB, Loucks et al. (2011) have advanced the concept of *energy availability* (EA; see Equation 3b). Energy availability is calculated as energy expended during exercise deducted from the dietary EI. The remaining energy is then available to support a range of physiological functions including the maintenance of cellular integrity, thermogenesis, immune function, growth, reproduction, locomotion and non-exercise physical activities.

Equation 3

a) $EB = EI - \text{total daily EE}$

b) $EA = EI - \text{exercise EE}$

Energy availability is proposed to be more useful than EB in managing the diet and exercise in athletes because changes in non-exercise EE can occur easily. As EA becomes restricted, physiological process can slow down, thereby reducing total daily EE and potentially bringing the body back into EB despite the restriction. In these circumstances, non-exercise EE can be difficult to quantify, particularly in the field. This has been demonstrated experimentally by Stubbs et al. (2004), who observed eight lean males living in a whole-room calorimeter for a week during caloric restriction. Although the dietary EI, and exercise EE were constant each day, their EB rose by approximately 90 kcal/d suggesting a compensatory slowing of physiological functions. It was estimated that after 3 wk of these conditions, the subjects would have been in EB, albeit a pathological state of EB as their EA would be reduced substantially.

Energy availability is expressed relative to fat free mass (FFM) as this component of body composition is more metabolically active than fat mass (FM). Young healthy individuals are thought to be in a state of EB when EA is ~ 45 kcal/kg FFM/d (Loucks and Thuma, 2003; Loucks et al., 1998; Mulligan and Butterfield, 1990) and BMR is ~ 30 kcal/kg FFM/d (Beidleman et al., 1995; Fogelholm et al., 1995; Mulligan and Butterfield, 1990; Myerson et al., 1991; Thompson and Manore, 1996; Wilmore et al., 1992). By measuring EA it can be determined whether an individual has sufficient energy to support resting physiological functions necessary to maintain health. The following EA guidelines have been developed for athletes with regards to weight management (Loucks et al., 2011).

- Attempting to reduce mass: >30 kcal/kg/FFM/d; <45 kcal/kg/FFM/d
- Attempting to gain mass: >45 kcal/kg/FFM/d
- Attempting to maintain weight: ~ 45 kcal/kg/FFM/d

Interestingly, when EA is reduced, particularly below ~ 30 kcal/kg/FFM/d, it has been suggested that RMR is reduced (Myerson et al., 1991) and reproductive function and bone

formation are suppressed (Loucks, 2003; Loucks and Thuma, 2003; Loucks et al., 2011). These shifts in energy distribution allow the body to conserve energy. While this may restore EB to facilitate survival, reproductive function and bone health may be compromised.

Reducing EA is an underpinning requirement for reducing mass. The two major complications associated with strategies designed to achieve this are as follows:

1. Feelings of hunger are unpleasant, stressful and difficult for athletes to suppress, ignore and thus sustain.
2. Chronic, excessive or poorly timed energy restriction can be harmful to an athlete's health and ability to perform.

2.8.1 Biomarkers of low energy availability

Early studies observed low triiodothyronine (T_3) in amenorrheic but not regularly menstruating female athletes (Loucks et al., 1992; Marcus et al., 1985; Myerson et al., 1991). Triiodothyronine is the major regulator of RMR, which constitutes a large component of total daily EE even in athletic populations. These early findings led Loucks and Callister (1993) and Loucks and Heath (1994) to measure thyroid function in female subjects exposed to four different levels of EA; 10, 20, 25, and 40 kcal/kg/FFM/d. Energy availability was controlled by manipulating diet and exercise together and independently (Loucks and Heath, 1994), but also by manipulating the intensity of exercise (Loucks and Callister, 1993). Regardless of how EA was restricted, thyroxine (T_4) and T_3 serum concentrations dropped dramatically when EA was equal to or less than 25 kcal/kg/FFM/d. Thyroid hormones may be suitable biomarkers for identifying when athletes fall below a threshold EA, below which physiological functions are impaired. Subsequent studies looked at hormones more directly involved with reproduction function.

Ovarian function is dependent on the frequency of luteinising hormone (LH) secretion by the pituitary. A series of studies have shown that when EA falls below 30 kcal/kg/FFM/d LH is suppressed, while amplitude is increased across 24 h of sampling in young, healthy, normally menstruating females (Loucks and Heath, 1994; Loucks and Thuma, 2003; Loucks et al., 1998). This occurred regardless of whether EA was restricted due to

exercise and diet independently or in combination. Similar observations have been made with biomarkers of bone turnover (Ihle and Loucks, 2004).

Using the same experimental design, Ihle and Loucks (2004) measured markers of bone formation (osteocalcin: OC and serum type I procollagen carboxy-terminal propeptide: PICP) and resorption (urinary N-terminal telopeptide: NTX) during an EA resulting in approximate EB (45 kcal/kg/FFM/d) and at three levels of restricted EA; 30, 20, and 10 kcal/kg/FFM/d. Changes in the NTX (attenuated bone resorption) were observed when EA restriction was quite extreme (10 kcal/kg/FFM/d). In contrast, OC and PICP were progressively reduced at all three levels of energy restriction, indicating impaired bone formation. It was concluded that the threshold for impaired bone formation occurs at a higher EA than the threshold for impaired bone resorption. In summary, both reproductive function and bone health are abruptly impaired when EA falls below ~30 kcal/kg/FFM/d. This is approximately equivalent to RMR and suggests that an EA below 30 kcal/kg/FFM/d fails to provide sufficient energy to maintain basic physiological functions.

2.8.2 Female Athlete Triad and Relative Energy Restriction in Sport (RED-S)

The *Female Athlete Triad (the triad)* is a medical condition that comprises the following three interrelated conditions (Nattiv et al., 2007; De Souza et al., 2014):

1. Low energy availability with or without disordered eating
2. Menstrual dysfunction
3. Low bone mineral density

Female athletes often present with one or more of these conditions which if left untreated can progress to more serious pathological states, including clinical eating disorders, amenorrhea and osteoporosis. De Souza et al. (2014) have published a consensus statement on treatment and return to play for the triad. It is important to emphasise that disordered eating in athletes exists on a continuum, starting with relatively healthy eating behaviours and dieting with occasional lowering of EI to promote weight loss. This can progress through to more extreme behaviours and chronic or excessive energy restriction. The continuum ends with clinical eating disorders, which are characterised by distorted

body image, weight fluctuations, abnormal eating behaviours and variable performance (Klungland Torstveit and Sundgot-Borgen, 2012).

Recently, an International Olympic Committee (IOC) work group has introduced the term *Relative Energy Deficiency in Sport* (RED-S), which suggests should provide an umbrella for the *Female Athlete Triad* (Mountjoy et al., 2014). *Relative Energy Deficiency in Sport* is defined as an energy deficiency relative to the balance between EI and the EE required for basic physiological functions to take place and homeostasis to be maintained. While *the triad* consists of the three components (EA, menstrual function and bone health), RED-S acknowledges that energy deficiency can result in a syndrome affecting an array of physiological functions. In addition to menstrual function and bone health, relative energy deficiency can have health consequences broadly described as metabolic, immunological, endocrine, haematological, growth and developmental, psychological, cardiovascular, and gastrointestinal. *Relative Energy Deficiency in Sport* also addresses the wide ranging effects that energy deficiency can have on performance, which include decreased endurance, increased injury risk, decreased training response, impaired judgement, decreased coordination, decreased concentration, irritability, depression, decreased glycogen stores and decreased muscle strength. Another major difference is that RED-S also addresses the fact that male athletes are affected by these outcomes whereas *the triad* is specific to females.

The prevalence of eating disorders in athletes has been reported by many researchers, with variable results. The prevalence of disordered eating has been estimated to be about 20% in elite female athletes and 8% in elite male athletes, although this varies greatly by sport (Martinsen et al., 2010; Sundgot-Borgen et al., 2013) and by the perceived performance improvements and sociocultural pressure to be light and lean (Byrne and McLean, 2002; Sundgot-Borgen and Torstveit, 2010). Athletes competing in sports described as *weight sensitive* are at an increased risk of developing eating disorders or disordered eating. Sundgot-Borgen et al. (2013) have described the following three categories of *weight sensitive* sports:

1. *Gravitational sports*

- These require the body to overcome the resistive forces of gravity in order to perform

- long-distance running, cross-country skiing, road and mountain bike cycling, ski jumping and jumping in athletics

2. *Weight-class sports*

- wrestling, judo, boxing, taekwondo, weight lifting, lightweight rowing

3. *Aesthetically judged sports*

- rhythmic and artistic gymnastics, figure skating, diving and synchronised swimming

Road cyclists fall into the first category as a *gravitational sport*. Endurance athletes, particularly female endurance athletes perceive a greater pressure to be lean and this is associated with a greater prevalence of eating disorders (Byrne and McLean, 2002). The prevalence of disordered eating in male cyclists has been reported to be approximately 50% (Ferrand and Brunet, 2004). It is expected that the prevalence of disordered eating and certainly low EA is significant amongst female road cyclists although this remains to be studied.

2.8.3 The interaction between participation in elite road cycling and bone health

Low BMD has been reported in endurance athletes, arising as a direct consequence of their sports participation (Scofield and Hecht, 2012). The BMD of cyclists has been found to be lower compared to other athletes (Rector et al., 2008) and non-athlete matched controls (Campion et al., 2010). In male cyclists, reductions in BMD of ~1.5% have been reported in a single cycling season (Barry and Kohrt, 2008) and Lombardi et al. (2012) have measured increases in biomarkers of bone resorption during a three-week stage race. Numerous other studies support the finding that cycling is a high risk sport for bone health for both males (Nichols et al., 2003; Olmedillas et al., 2011) and more recently for females (Sherk et al., 2014). The consistent finding of poor bone health in cyclists may be due to a lack of weight-bearing activity (Milgrom et al., 2000) as well as the low EA (Loucks et al., 2011) resulting from weight loss practices and/or the high volume of exercise EE (Barry and Kohrt, 2008). Given these compounding risk factors, it is important to identify risk factors for impaired bone health that are potentially modifiable.

2.8.3.1 Biomarkers of bone turnover

Bone consists largely of an extracellular matrix composed of proteins and hydroxyapatite crystals. There are three types of cells that continually remodel bone: osteoblasts (involved in bone formation); osteoclasts (involved in bone resorption); and osteocytes (transfer mineral from inside the bone to the surface). Osteocytes are interconnected allowing the transfer of Ca^{2+} between cells from the inside of cortical bone to the surface (osteocytic osteolysis).

There has been interest in the contribution of dermal calcium losses (sweat calcium loss) during cycling to poor bone health (Barry and Kohrt, 2008; Barry et al., 2011; Guillemant et al., 2004). The acute and significant dermal calcium losses during exercise may cause a decline in serum ionised calcium concentrations during exercise. Calcium has a number of important physiological functions and serum calcium concentration is therefore defended vigorously within the body. Bone is the body's largest reservoir of calcium so reductions in serum ionized calcium are replaced by demineralization of bone. Parathyroid hormone (PTH) is released by the parathyroid gland and acts on the PTH receptors located on the bones and kidneys. Within the bone, PTH indirectly stimulates osteoclast activity thereby increasing bone resorption and serum ionized calcium concentration. Therefore, PTH is a biomarker of bone resorption.

The extracellular matrix of bone consists of proteins formed by the osteoblasts. Approximately 90% of the extracellular matrix of bone is Type I collagen, a helical protein that is cross-linked at the N- and C- terminal ends (Boron and Boulpaep, 2012). Other biomarkers of bone resorption include Cross linked C-telopeptide of type I collagen (CTX-I). The cross linked C-telopeptides are found in a number of tissues but particularly in bone. These peptides are cleaved by osteoblasts and are therefore associated with bone resorption. Cross linked C-telopeptide of type II collagen (CTX-II) was originally proposed as a marker of cartilage degradation (Christgau et al., 2001), although a recent investigation by van Spil et al. (2013) has shown CTX-II to correlate more strongly with other markers of bone resorption. Amino-terminal cross-linking telopeptides of type I collagen (NTX-I) has been used as a biomarker of bone resorption, measured either in urine or serum and decreases in response to anti-resorptive therapies in osteoporotic adults (Civitelli et al., 2009).

Markers of bone formation include Procollagen I N-terminal propeptide (PINP) and Procollagen type I C-terminal propeptide (PICP). These peptides are cleaved from procollagen molecules before they assemble into fibrils (Civitelli et al., 2009). Procollagen type I C-terminal propeptide can be regulated by growth hormone, and thyroid hormones making it less specific to bone formation. Another biomarker of bone formation is OC; a bone matrix protein formed by osteoblasts. Osteocalcin is incorporated by osteoblasts into the bone matrix, however; a small portion is released into circulation (Riggs et al., 1986). The measurement of circulating OC is therefore proportional to bone formation. Osteocalcin has a high circadian and biological variability complicating its interpretation (Seibel, 2005). Finally, bone specific alkaline phosphatase (bone ALP) is an enzyme found on the cell membrane of osteoblasts and is required for bone mineralization (Harris, 1990). This too can be measured from serum. While beyond the scope of this review, there are several biomarkers of bone resorption and formation which have varying degrees of specificity, sensitivity and ease of measurement (Civitelli et al., 2009; Clowes et al., 2002; Eastell and Hannon, 2008; van Spil et al., 2013). Much of the literature in this area relates to osteoporosis in general populations and the response of osteoporotic adults to therapies.

2.8.3.2 Techniques for measuring biomarkers of bone turnover

Most of the discussed biomarkers of bone turnover can be measured from serum using either the radioimmunoassay technique (RIA) or the enzyme-linked immunosorbent assay (ELISA). Serum is the component of blood that does not contain blood cells or clotting factors (fibrinogens). It consists of all proteins not involved in coagulation as well as all the electrolytes, antibodies, antigens, hormones and any other exogenous substance. To obtain serum, whole blood is collected into a tube and allowed to stand at ambient temperature until the blood has clotted. The clot is removed by centrifugation, leaving the serum as the supernatant. This is transferred into a polypropylene tube and if not analysed immediately, apportioned into ~ 0.5 ml aliquots, frozen and stored for later analysis.

The radioimmunoassay technique is a sensitive assay technique used to measure the concentration of antigens such as hormones in the serum. This technique takes advantage of immune function whereby certain antibodies have a high affinity for specific antigens. With RIA, a known concentration of the antigen of interest is made radioactive

and then mixed with a known amount of the antibody for that antigen. The collected sample with an unknown concentration of the antigen is then also added. The radioactively labelled antigen competes with the unlabelled antigen for antibody binding sites until equilibrium is reached. The higher the concentration of unlabelled antigen in the collected sample, the more the radioactive antigen is displaced, thereby decreasing the ratio of antibody-bound radiolabelled antigen to free radiolabelled antigen. The unbound antigens are separated from the bound antigens and the radioactivity of the unbound antigens is measured using a gamma counter. Known standards are used to create a standard displacement curve and an equation for the relationship between unlabelled antigen concentration and radioactivity is derived. This allows accurate calculation of a range of antigen concentrations from collected samples. It is possible to obtain antibodies that are highly specific and have a high affinity for the chemical structure (antigen) of interest. This allows detection of minute amounts of a specific antigen in blood. Radioimmunoassays can be used to measure most hormones as well as drugs, viruses and toxins (Boron and Boulpaep, 2012). The RIA does involve radioactive substances, requiring special precautions and licensing for those handling these substances. Recent adaptations substitute radioactivity for chemiluminescent or enzymatic detection.

Another common technique for serologic testing is the *enzyme linked immunosorbent assay* (ELISA). There are three forms of ELISA: 1) *indirect* ELISA; 2) *sandwich* ELISA; and 3) *competitive* ELISA. With the indirect ELISA, serum is transferred into wells of a microtiter plate that are pre-coated with the antigen of interest. If antibodies specific for that antigen are present in the serum, they will bind to antigen in the wells. The wells are then washed out, removing unbound primary antibodies from the sample. A solution of a secondary antibody against the primary antibody is added to the wells. The second antibody is covalently conjugated to an enzyme; it is *enzyme-linked*. The wells are again washed to remove any unbound enzyme-linked antibody. Finally, a colourgenic substrate for the enzyme is added to the wells. This reacts with the enzyme linked to the secondary antibody generating a visible colour that can be measured with an electronic plate reader. Like RIA, known concentrations of the primary antibody for the antigen of interest are used to create a standard curve. The equation derived from this curve is used to determine the concentration of the antigen of interest in the sample. The technique is indirect because the test measures the antibody for the antigen of interest rather than directly measuring the antigen of interest.

With the *sandwich* ELISA, the surface of the well is pre-coated with antibodies for the antigen of interest. Again, serum is added to the wells and the antigen of interest binds to the antibodies on the well surface. The well is washed and an *enzyme-linked* antibody against the antigen is added. The well is washed, and a colourgenic substrate is added. The antigen of interest becomes *sandwiched* between the antibody on the surface of the wells and the *enzyme-linked* antibody; hence the name, *sandwich* ELISA.

With the competitive ELISA, serum is first transferred to a tube containing the antibody against the antigen of interest. The antibody-antigen solution is then added to the well which is pre-coated with the antigen. Some of the antibody from the original solution will be unbound and will therefore bind to the antigen on the well surface. The well is then washed and a secondary *enzyme-linked* antibody against the primary antibody is added. Again, a colourgenic substrate is added and the final colour compared to the standard curve. Interpreting the results of a *competitive* ELISA differs to the other ELISA tests in that lower colourgenic reactions indicate greater concentrations of the antigen of interest in the original serum sample. The different ELISA tests are designed specifically for different types of antigens of interest. The manufacturer of the ELISA kits typically provide all the necessary pre-coated wells and reagents as well as highly specific instructions which must be followed carefully to ensure accuracy and reliability.

2.9 Strategies used to achieve an energy deficit

There is an almost infinite number of ways to achieve an energy deficit by manipulating either side of the EB equation. For female cyclists, a low BW is desirable for performance but currently there is no best practice for achieving this goal and sometimes athletes and coaches lack evidence based guidance. As with any intervention prescribed to an athlete, achieving an energy deficit for the purpose of reducing BW should be done in a manner that meets the following criteria:

- Effective
 - reduces fat mass with minimal impact on functional lean mass
- Healthy
 - does not impair physiological functions as described with RED-S

- Sustainable
 - can be sustained for durations sufficient to elicit the desired change with excessive physiological or psychological stress
- Logistically sound
 - can be applied practically in the field

The issue of sustainability is multifaceted. It is desirable that weight loss strategies achieve an energy deficit in a way that is psychologically and physiologically sustainable, retain energy availability at a healthy level, and partitions the loss of BW to fat and non-functional lean mass. This can be achieved by manipulating an individual's daily energy flux, the timing of their EE and EI, and the macronutrient composition of their diet. These areas present a host of possible strategies that could be used to manipulate body composition in ways that satisfy the above stated criteria.

2.9.1 High and low energy flux strategies

A negative EB can be achieved by increasing exercise EE while simultaneously maintaining an EI slightly below EE. An identical energy deficit and reduction in EA can be achieved by having a low to moderate exercise EE while simultaneously reducing EI below EE. Broadly, the following two options are available for athletes seeking to achieve an energy deficit:

- High Energy Flux (HEF)
 - High volume/intensity training with moderate dietary intake; or
- Low Energy Flux (LEF)
 - Moderate volume/intensity training with low dietary intake.

Although each approach reduces EA identically at first, it is possible that the adaptive responses may affect physiological functions such as metabolic rate change, health outcomes and the ensuing energy deficit (i.e. a reduction in EE caused by a reduction in RMR will reduce the energy deficit although EA remains constant) in different ways. A number of studies have been designed to compare these strategies with the purpose of identifying the independent effects of altering exercise and/or diet to create low EA on

reproductive (Loucks et al., 1998) and thyroid (Loucks and Callister, 1993) function, as well as Leptin responses (Hilton and Loucks, 2000) in females. The results of these studies showed that a large energy deficit has profound effects on physiological and metabolic function regardless of how it is achieved. The *exercise stress hypothesis* has therefore been refuted (Loucks et al., 1998) and replaced with the *energy availability hypothesis*. The latter surmises that gonadotropin-releasing hormone (GTRH) and thus luteinizing hormone (LH) are disturbed not solely by high levels of physical activity, but rather by the stress of having insufficient EI to match the EE demands of physical activity. It is not the stress of exercise or restricted EI alone but the reduced EA that impairs reproductive and bone health. As long as EA is maintained above 30 kcal/kg/FFM/day, it seems likely that health can be largely preserved while BW is reduced regardless of whether this is brought about by a HEF or LEF strategy.

Exercise has been shown to suppress subjective ratings and hormonal markers of appetite (Cheng et al., 2009). This observation supports the theory that a greater volume of training (HEF) may make the energy deficit more sustainable. One difficulty encountered by athletes is *mindless eating* or *non-essential eating* which occurs out of boredom or for reasons other than an increase in appetite. The higher training volume associated with a HEF could reduce the time void an athlete can fill by eating. By reducing EI for a LEF strategy, it may be difficult to incorporate sufficient micronutrients to maintain a healthy diet and support quality training. Conversely, the increased training volume associated with a HEF may increase the risk of over-training or injury. There is clearly a need for additional research into the efficacy of these strategies for populations of elite athletes. Until then, a conservative approach which lies somewhere in the middle of a high and low energy flux may be prudent.

2.9.2 Meal frequency

A higher meal frequency or *grazing* as opposed to a low meal frequency or *gorging* may help reduce body fat. While the topic has received a great deal of attention in general, aged, and overweight / obese populations, the theory has not been investigated thoroughly in athletes (La Bounty et al., 2011). One theory is that the thermic effect of food (TEF) is increased by increasing meal frequency, thereby increasing total daily EE. However, Kinabo and Durnin (1990) and Smeets and Westerterp-Plantenga (2008) showed no significant differences in the TEF (indirect calorimetry) for diets of differing meal frequency

and. An increase in 24 h fat oxidation, a reduction in 24 h glucose oxidation and greater satiety were observed when consuming the higher frequency of meals (Smeets and Westerterp-Plantenga, 2008). The International Society of Sports Nutrition Position Stand on Meal Frequency (La Bounty et al., 2011), concluded that meal frequency does not appear to affect metabolic rate.

There is a likely interaction between macronutrient intake and meal frequency which should be considered, especially when manipulating periodicity of eating. Moore et al. (2009) have shown that muscle protein synthesis has a dose response to dietary protein following resistance exercise which reaches an optimal rate at ~20 g (0.3 g/kg). Post-exercise protein consumed in quantities less than 20 g may produce a sub-optimal stimulus for protein synthesis. When increasing meal frequency, it may be important to ensure that meals within close proximity to exercise contain sufficient protein and that this intake is not simply diluted into a series of smaller portions throughout the day (Areta et al., 2013).

While the effects of meal frequency on BW and body composition have conflicting results (La Bounty et al., 2011), a higher frequency of feeding has been associated with better appetite control. Speechly et al. (1999) and Speechly and Buffenstein (1999) fed subjects a *pre-load* of 1/3 of their daily energy requirement as one meal or five meals administered hourly through the first half of the day. Those who had five meals exhibited greater appetite control (27% lower EI at lunch) and had lower ratings of hunger, appetite and urge to eat. Stote et al. (2007) and Smeets and Westerterp-Plantenga (2008) have also shown that hunger was augmented and satiety improved by consuming three meals compared to one or two respectively. Cameron et al. (2010) examined more common eating patterns; three meals versus three meals plus three snacks for the same energy deficit. Using this model, obese individuals had no significant differences in subjective ratings of hunger or fullness between meals, and no differences in gut hormones (ghrelin and neuropeptide YY) that stimulate hunger. While there are some inconsistencies in the literature, it appears as though a higher frequency of meals (three vs. one or two) induces greater appetite control, however, Cameron et al. (2010) found no benefit in combining snacks with three meals.

Research into meal frequency and timing of EI or EB fluctuations in athletes is scarce. The few studies that exist seem to support the use of higher frequency of meals. Iwao et

al. (1996) focussed on body composition of boxers in a hypocaloric state for 2 wk. One group consumed two meals per day while the other consumed six. While there was no difference in weight loss, the high frequency group had reduced catabolism of lean mass and had a greater reduction in skinfolds. It could be speculated here that the higher frequency of meals also introduced sufficient protein in close enough proximity to exercise so that protein breakdown was attenuated.

Benardot (2005) monitored 60 collegiate athletes, providing them with a 250 kcal snack to consume following breakfast, lunch and dinner. Compared to a control group that consumed a non-caloric placebo, the experimental group experienced a reduction in fat mass (-1.03%) and a gain in lean mass (+1.2 kg). When all the snacks were consumed (750 kcal), total daily EE increased by only 128 kcal suggesting caloric intake at main meals (breakfast, lunch and dinner) was reduced.

Analysing meal frequency only considers the EI and by not including EE, studies ignore the flux of EB throughout the day. Deutz et al. (2000) adopted a unique approach and calculated EI and EE for each hour in the day such that the magnitude and frequency of energy deficits and surpluses could be determined. These data were then compared to body composition measured using DXA and skinfolds in a cohort of female gymnasts and runners. Interestingly, there was a positive relationship between the number of daily deficits >300 kcal and fat mass. There was also a negative correlation between the number of energy surpluses >300 kcal and fat mass. The authors suggest delayed or restrained eating should be avoided by athletes trying to reduce or maintain fat mass, hypothesising that restrained eating results in compensatory reductions in BMR, thereby reducing total daily EE. While Deutz et al. (2000) present findings that should be investigated further, their measures of EE and EI may need refining. Their measures of EE were based around indirect estimates of RMR which particularly for young athletes, who may still be growing, may increase the risk of error. Similarly, their estimates of EI, like many other self-report measures (Poslusna et al., 2009), may have resulted in under-reporting.

Evidence from the small number of studies in athletic populations (Benardot, 2005; Deutz et al., 2000; Iwao et al., 1996) would suggest that at least three meals and three snacks should be consumed throughout the day to maintain satiety and avoid a catabolic state. Protein in the form of a 20 g bolus is beneficial following exercise and at 3-4 other eating

occasions per day, rather than evenly distributing to a series of smaller boluses throughout the day or relying on several large serves (Areta et al., 2013). It may also be important to limit the frequency and magnitude of energy deficits throughout the day (Deutz et al., 2000). These strategies may have favourable outcomes on body composition in athletes in sports where being lean is important although it is clear that further investigation is required.

2.9.3 Energy density and macronutrient composition of the diet

While it is simple to talk only about total EI, the macronutrient composition (CHO, fat and protein) and the energy density of foods has also proven to have an impact on satiety and satiation. This in turn can affect EI and therefore the efficacy of nutritional practices designed to manipulate or maintain body composition. Energy density (ED) refers to the amount of energy (calories or joules) per gram of food. The energy content of fat is about ~9 kcal/g while that of proteins and carbohydrates are ~4 kcal/g. Typically, foods high in fat have a greater ED than those higher in proteins and carbohydrates. Foods with high water content have a lower ED as water contributes to the weight but not energy. Therefore, even foods that are high in fat but also high in water content can have a lower ED. Assuming an individual eats the same volume of food each day, even small changes to total ED can have a significant impact on total daily EI (Rolls et al., 1999a).

Adding water to foods to increase volume and reduce the ED increases satiety (Rolls et al., 1998), however, the water must be incorporated into the food and not simply consumed as a beverage to get this effect (Rolls et al., 1999b). A possible explanation is that the rate of gastric emptying 30 – 60 min post meal is positively related to the ED, such that low ED foods are passed from the stomach to the duodenum more slowly. The perceived pleasantness or the sensory-specific satiety of a food declines as it is consumed. In a study where ED of a milk-based liquid food was manipulated independently of macronutrient composition, food volume affected the cessation of eating more so than did energy content (Bell et al., 2003).

Factors other than ED must also be considered. Studies have used soups and beverages because manipulating the ED with water is easy. Whether the food exists in a solid or liquid form may impact on satiety. Fruits and vegetables lend themselves to such research as they can be pureed without changing the ED. Whole fruit has been shown to have

greater satiating effects than pureed fruit or juice of the same volume and ED (Flood-Obbagy and Rolls, 2009).

Some studies have shown that manipulating the macronutrient content of foods alters *ad libitum* EI. Lissner et al. (1987) and Kendall et al. (1991) have compared high, moderate, and low fat diets of similar palatability and appearance in which consumption was *ad libitum*. Energy intake was reduced in the low fat diet and resulted in weight loss in the female subjects. As indicated by Rolls (2009), macronutrient manipulation simultaneously altered ED in these studies. To differentiate between the effects of macronutrients and ED, foods must be diluted with water or incorporated with water rich vegetables or fruits. Saltzman et al. (1997) increased fat content while maintaining ED, fibre content and palatability of foods and found that increasing fat content from 20 to 40% of daily intake had no significant impact on EI in twins. Rolls et al. (1999a) independently manipulated ED and fat content to create four, 4 d diets for a group of obese and lean women and confirmed that ED, but not fat content, affects total daily EI. Based on these findings, there is a strong rationale for manipulating the ED of diets for athletes wishing to manipulate body composition. It appears likely that reducing ED could assist in meeting the previously described criteria outlined for a suitable strategy to reduce EA.

2.9.4 Summary of strategies to achieve an energy deficit

This section has identifying the following three broad areas within which EA can be manipulated:

- 1) Energy flux
 - the total energy expenditure and intake can differ dramatically, yet result in the same energy availability
- 2) Timing and frequency of energy intake
 - The scheduling of meals and the frequency with which total energy intake is partitioned throughout the day
- 3) Energy density
 - The energy content per gram of food consumed can be manipulated by macronutrient and water content

The literature investigating these concepts and strategies in combination or isolation in athletic populations is scarce. With that said, the existing literature would support the use of increased meal frequency that is timed to provide EI in close proximity to exercise. High biological value proteins should be consumed in ~20 g serves to stimulate protein synthesis. To improve satiety and reduce the psychological strain associated with restricted EI, the ED of food may be reduced. Again, sport scientists and researchers working with athletic populations should ensure that interventions aimed at reducing EA are effective, healthy, sustainable and logistically sound.

2.10 Summary of the Literature Review

To measure body composition in female cyclists, a number of techniques can be used. Based on the resources available and accuracy of the techniques reviewed, anthropometry and DXA are those most appropriate for monitoring athletes in our laboratory. Body composition must be measured accurately to determine the energy requirements of athletes and the efficacy of strategies used to manipulate body composition.

Changing EA is required to affect change in BW. To accurately do this, EI and/or EE must be altered and to do this purposefully, accurate measurements of these variables are required. Energy intake can be measured using self-report techniques and the reliability of these can be improved with education on food weighing and quantifying portion sizes. Energy expenditure can be measured in the field using power meters, heart rate monitors and motion sensors while the *gold standard* for validating these techniques is doubly labelled water. Under laboratory conditions, indirect calorimetry is the most practical, particularly when quantifying EE during exercise.

Energy restriction can be achieved through an immeasurable number of techniques, involving the manipulation of energy flux, timing and frequency of EI and the ED of foods consumed. The interplay between exercise and diet is complicated and there are few investigations that have examined strategies for manipulating body composition in elite female cyclists that are effective, healthy, sustainable and logistically sound. Nonetheless, some basic recommendations can be drawn from studies into general populations and applied to athletes.

This thesis will focus on improving our understanding of female cyclists' perceptions towards body composition and how it relates to performance; the physiques that perform at different ability levels in women's cycling will be assessed and techniques that can be used to quantify EE in road cyclists will be trialled. The concept of functional lean mass is introduced by investigating lean mass power output relationships, and then presenting a case where attempts are made to manipulate lean mass and fat mass in a female cyclist. Finally, a strategy that may improve bone health while meeting other nutritional targets is investigated through a dietary intervention.

CHAPTER 3: RACE WEIGHT: PERCEPTIONS FROM ELITE FEMALE ROAD CYCLISTS

3.1 Introduction

A high power output to body weight (BW) ratio (W/kg) is desirable for performance in road cycling and higher performing female Australian cyclists have been shown to be lighter and leaner than their less successful team mates (Martin et al., 2001). Indeed, a working group of the International Olympic Committee Medical Commission recently confirmed the identity of cycling as a weight-sensitive sport, noting that a high BW “*restricts performance because moving the body against gravity is an essential part of the sport*” (Sundgot-Borgen et al., 2013). While there are no controlled prospective studies investigating risk factors for disordered eating in athletes, participation in weight-sensitive sports is believed to increase the risk of unhealthy practices used to manipulate body composition. Previous research has shown that endurance athletes, particularly female endurance athletes perceive a greater pressure to be lean and this is associated with a greater prevalence of eating disorders (Byrne and McLean, 2002). While no known studies have investigated these themes specifically in female cyclists, male cyclists have been reported to have a relatively high prevalence of disordered eating behaviours (Filaire et al., 2007; Riebl et al., 2007).

Although there is often a need to manipulate diet and training in order to achieve a competitive body composition in cycling, our experience at the Australian Institute of Sport has made us aware of case histories in which Australian (Lane, 2012) and International (Holcombe, 2012) female cyclists have resorted to “extreme” and potentially unhealthy weight-loss methods to achieve what they believe is an ideal BW for performance. These weight-loss practices increase the risk of creating an energy deficiency which may result in impaired reproductive function and bone status (Loucks et al., 2011), an increased prevalence of illness (Hagmar et al., 2008) and injuries (Rauh et al., 2010) and a reduction in athletic performance (Vanheest et al., 2014). More recently, the concept of relative energy deficiency in sport (RED-S) has been introduced, which emphasises a more complex interaction between excessive or prolonged energy deficiency, health and performance in both male and female athletes (Mountjoy et al., 2014). In addition to a range of mechanisms impairing performance, the RED-S concept describes a wider range of health consequences broadly categorised as immunological, reproductive, bone health,

endocrine, metabolic, haematological, growth, psychological, cardiovascular and gastrointestinal (Mountjoy et al., 2014). It is apparent that athletes exposed to frequent or prolonged periods of energy deficiency are at an increased risk of a broad range of health consequences. It is of interest to better describe the perceptions towards BW of athletes participating in weight conscious sports such as cycling.

The primary objective of this study was to describe the weight consciousness of elite female road cyclists. The secondary objective was to describe the BW satisfaction of female cyclists in the context of performance at a major competition. Self-reported seasonal fluctuations in BW and the methods used to manipulate BW were also evaluated, as was the association between BW manipulation and menstrual dysfunction.

3.2 Methods

Thirty-seven female cyclists who competed in the Road Race at the 2013 Australian National Road Cycling Championships (n = 32) and Oceania Championships (n = 5) volunteered to participate in this study after being approached by regional and state coaches that supported this research theme. Both races included multiple laps of a ~10 km circuit with a significant climb totalling ~1500 ascent metres. These races were selected as they attract the highest level of Australian cyclists and the course profiles are not dissimilar to those typically used in Union Cycliste Internationale (UCI) World Cup and World Championship Road Races. The study was approved by the Human Research Ethics Committee at the Australian Institute of Sport and all subjects were informed of testing protocols and risks of the study before providing written informed consent.

In the week leading up to competition (1-5 d prior to race day), cyclists were asked to complete the Female Cyclist Weight Management Questionnaire (FCWM) which consists of 19 questions (see Appendix A) that focus on the following themes: the level of the athlete (professional or amateur); the cyclist type (climber, sprinter, time trialist, all-rounder); their current actual BW (current BW) and how much it has fluctuated during their career since they stopped growing; whether they feel their current BW is ideal for performance in these races and if not, how much they would like to change it (ideal BW); whether they have attempted to change their BW in the past 12 mo and whether they were successful; how effective they perceive racing at their lowest weight to be; how frequently they are conscious about wanting to change their BW (10 point scale); what techniques

they have used to manipulate their BW in the past; whether they think their coach has ever been dissatisfied with their BW; and the regularity of their menstrual cycle.

Three questions related to BW reduction techniques (*“I avoid foods high in sugar”, “I vomited after eating”, “I used weight loss supplements or medications”*) have been adapted from The Eating Attitudes Test (Garner et al., 1982), while those related to menstrual function are adapted from the HUNT-2 Questionnaire (Holmen et al., 2003). The majority of the questions were designed specifically for this study and have not been used previously. It is acknowledged that this questionnaire has not been assessed for validity or reliability. The questionnaire was designed for pilot work and the researchers felt that at the competitions where it was used, it would be inappropriate to burden the athletes with completing further questionnaires due to time constraints and the proximity to competition.

3.2.1 Statistical analysis

Self-reported BW was investigated using linear mixed-effects modelling fit by restricted maximum likelihood estimation (REML) with BW context (current BW, career lowest BW, career highest BW and ideal BW) as a main effect and subject as a random effect. The interaction between cyclist type (climber, sprinter, time trialist, all-rounder) and BW context (current BW and ideal BW only) was investigated using a separate model with cyclist type and BW context as main effects and subject as a random effect. Body mass index [BW (kg) / height (m)²] was estimated based on reported current BW (current BMI), ideal BW (ideal BMI), lowest BW (lowest BMI) and highest BW (highest BMI).

The difference between current BW and ideal BW was expressed for all cyclists as a percentage of current BW (weight $\Delta\%$). The weight $\Delta\%$ was investigated using linear mixed-effects modelling fit by REML, with cyclist type and professional status (professional and amateur cyclists) as main effects and subject as a random effect.

Pearson's chi-squared test was used to assess differences between professional and amateur cyclists for responses towards perceptions of career lowest weight on performance, prevalence of eating disorders, coach dissatisfaction with weight and whether weight had been manipulated in the previous 12 mo. Fisher's exact test was used to identify any differences in the frequency of weight consciousness by Professional Status

and by best National Road Series (NRS) race ranking (Top 3; 4-10; 11-20; >20). The magnitude of BW change in the previous 12 mo was investigated using a linear mixed-effects model fit by REML with cyclist type and professional status as main effects and subject as a random effect.

The frequency of weight consciousness was dichotomised to 'once or more weekly' or 'less than once weekly'. A Pearson's chi-squared test was used to determine if there were differences in the frequency of weight consciousness depending on menstrual function; normal or dysfunctional (amenorrhoeic or oligomenorrhoeic). A linear mixed-effects model fit by REML was used to determine whether the magnitude of BW change differed in these groups. Tukey's HSD post-hoc analysis was used to determine the level of the difference for all mixed models. All statistical analyses were conducted using JMP Pro® v10 (SAS Institute Inc., Cary, NC, USA). An alpha level of 0.05 was used.

3.3 Results

3.3.1 Subject characteristics

Thirty seven female cyclists completed the survey (range 18 – 36 y; mean \pm SD 58.4 \pm 5.9 kg; 170 \pm 7 cm). This represents 32 (45%) of 71 starters at the National Championships and 5 at the Oceania Championships who had not already completed the survey at National Championships. Fifteen cyclists classified their status as racing for a professional team internationally in the last 12 mo and the average duration of racing as a professional was 1.6 \pm 0.8 y. The remaining 22 cyclists reported racing only domestically in the past 12 mo (amateur). Of the 22 amateurs, 12 cyclists had achieved a top 3 placing in a NRS race in the previous 12 mo, 6 achieved a 4-10 placing, and 4 did not achieve a top 10 place. The cyclists classified themselves as climbers n = 11; sprinters n = 6; time trialists n = 4; or as all-rounders n = 16.

3.3.2 Perceptions of the effect of body weight on performance

Twenty three cyclists (62%) reported that their current BW was not optimal for performance in these races. This did not differ between professional and amateur cyclists ($X^2_{1, 37} = 0.84$; p = 0.36). The analysis of self-reported BW revealed main effects for BW context ($F_{3, 107} = 88.76$; p < 0.01; see Table 5). Current BW was significantly higher than

lowest BW (mean [95% confidence interval]; 2.3 kg [1.4, 3.2]; $p < 0.01$) and ideal BW (1.6 kg [0.6, 2.5]; $p < 0.01$), but was lower than the highest BW (-3.0 kg [-3.9,-2.1]; $p < 0.01$). The difference between lowest BW and ideal BW was not significant (-0.7 kg [-0.2, 1.7]; $p = 0.16$). Current BMI was estimated to be (mean \pm SD) $20.3 \pm 2.2 \text{ kg}\cdot\text{m}^{-2}$ ($n = 7 \leq 18.5 \text{ kg}/\text{m}^2$); lowest BMI was $19.5 \pm 2.2 \text{ kg}/\text{m}^2$ ($n = 13; \leq 18.5 \text{ kg}/\text{m}^2$); ideal BMI was $19.8 \pm 1.9 \text{ kg}/\text{m}^2$ ($n = 10 \leq 18.5 \text{ kg}/\text{m}^2$) and highest BMI was $20.3 \pm 2.2 \text{ kg}/\text{m}^2$ ($n = 7 \leq 18.5 \text{ kg}/\text{m}^2$).

Table 5. Comparison of self-reported body weight changes and perceived ideal weight for performance

<i>Body weight context</i>	\bar{X} [95% CI]
<i>Current weight</i>	58.3 [56.4, 60.2] ^{L,H,I}
<i>Career lowest weight</i>	56.0 [54.1, 57.9] ^{C,H}
<i>Career highest weight</i>	61.3 [59.4, 63.2] ^{C,L,I}
<i>Ideal weight</i>	56.8 [55.2, 58.5] ^{C,H}

Results presented as means (\bar{X}); confidence intervals (CI). Significantly different ($p < 0.05$) to current weight (C), lowest weight (L), highest weight (H), ideal weight (I).

Table 6. Self-reported current body weight for different types of cyclists and the body weight perceived to be ideal for performance at major competition

<i>Cyclist Type</i>	<i>N</i>	<i>Current weight (kg)</i>	<i>Ideal weight (kg)</i>	Δ <i>Weight (%)</i>
		\bar{X} [95% CI]	\bar{X} [95% CI]	\bar{X} [95% CI]
<i>Climbers</i>	11	53.1 [50.4, 55.8] ^{S,T,A}	52.8 [50.1, 55.5]	0.5 [-0.7, 1.8] ^{S,T}
<i>Sprinters</i>	6	62.6 [58.9, 66.3] ^C	60.0 [56.3, 63.7]*	4.1 [2.4, 5.9] ^C
<i>Time trialists</i>	4	62.5 [58.0, 67.0] ^C	59.5 [55.0, 64.0]*	4.7 [2.5, 6.8] ^C
<i>All-rounders</i>	15	59.3 [57.0, 61.5] ^C	57.8 [55.4, 59.9]*	2.6 [1.5, 3.7]
<i>All Cyclists</i>	37	58.3 [56.4, 60.2]	56.8 [55.2, 58.5]*	2.5 [1.6, 3.3]
<i>Professionals</i>	15	57.6 [54.5, 60.6]	56.2 [53.6, 58.8]*	2.2 [0.9, 3.5]
<i>Amateurs</i>	21	58.8 [56.3, 61.4]	57.3 [55.1, 59.5]*	2.7 [1.5, 3.8]

Results presented as means (\bar{X}) and 95% confidence intervals (CI).

*Ideal weight significantly ($p < 0.05$) different to current weight.

Significantly different ($p < 0.05$) to All-rounders ^A, Climbers ^C, Sprinters ^S, Time Trialists ^T.

As shown in Table 6, there was an interaction between cyclist type and BW context when analysing only current BW and ideal BW ($F_{3,33} = 6.15$; $p < 0.01$). Compared to climbers, current BW was significantly higher for sprinters (9.5 kg [2.2, 16.7]; $p < 0.01$), time trialists (9.4 kg [1.1, 17.7]; $p = 0.02$) and all-rounders (6.2 kg [0.6, 11.8]; $p = 0.02$). There were no significant differences between sprinters, time trialists, and all-rounders ($p > 0.05$). While there was a trend towards ideal BW being lower for climbers compared to sprinters (-7.2 kg [-0.1, 14.4]; $p = 0.05$) there were no statistically significant differences between ideal BW for different types of cyclists. Current BW was greater than ideal BW for sprinters (2.6 kg [0.8, 4.3]; $p < 0.01$), all-rounders (1.6 kg [0.5, 2.8]; $p < 0.01$) and time trialists (3.0 kg [0.8, 5.1]; $p < 0.01$), but not for climbers (0.3 kg [-1.0, 1.6]; $p = 0.99$).

There were no main effects for professional status on weight $\Delta\%$ ($F_{1,28} = 0.43$; $p = 0.514$) so it was dropped from the model. Cyclist type had significant main effects on weight $\Delta\%$ ($F_{3, 32} = 5.92$; $p < 0.01$; see Table 6). Compared to climbers, the weight $\Delta\%$ was significantly greater for sprinters (3.6% [0.7, 6.5]; $p < 0.01$) and time trialists (4.1% [0.8, 4.2]; $p = 0.01$) with a similar trend compared to all-rounders (2.1% [-0.01, 4.4]; $p = 0.007$).

When asked how being at their career lowest BW affects their performance, 10 (27%) of the 37 cyclists responded saying it was not beneficial. More specifically, 2, 3 and 5 athletes reported that it was '*extremely harmful*', '*somewhat harmful*' or had '*no effect*' respectively. Twenty cyclists (54%) reported that their career lowest BW was '*beneficial*' and 7 (19%) that it was '*extremely beneficial*' for performance respectively. There was no significant difference between amateur and professional cyclists' responses to this question ($\chi^2_{4, 37} = 7.77$; $p = 0.08$). All but one athlete reported a belief that "*female cyclists are a weight conscious population*". Twelve cyclists (32%) reported that their coach or sports director had "*expressed dissatisfaction with their BW at some point in past 12 months*" and this response was more frequent with the professional cyclists ($n = 8$; $\chi^2_{1, 37} = 5.029$; $p = 0.03$).

3.3.3 Body weight changes and manipulation

Twenty six (70%) cyclists reported attempting to reduce their BW in the past 12 mo. Twenty four reported reducing BW by an average (\pm SD) of 2.4 ± 1.0 kg (range; 1 - 5 kg). This represented $4.2 \pm 1.8\%$ of the current BW (range; 1.7 - 9.8%). There was no statistically significant difference between professional and amateur cyclists ($\chi^2_{1, 37} =$

0.509; $p = 0.48$). Five cyclists (14%) reported having been previously diagnosed by a physician as having an eating disorder. This was no more prevalent in professionals than amateurs ($\chi^2_{1, 37} = 0.001$; $p = 0.98$). There were no main effects for cyclist type ($F_{3,18} = 1.75$; $p = 0.19$) or professional status ($F_{1,18} = 0.021$; $p = 0.89$) on the magnitude of BW change in the previous 12 mo. These findings persisted when the magnitude of BW change was expressed as a percentage of current BW ($P > 0.05$).

Responses to the question “*In the last 12 months, how often were you conscious about wanting to change your body weight?*” are shown in Figure 2. Sixteen (43%) cyclists reported being conscious of this less than once a week while 20 (54%) reported being conscious of this once a week or more frequently. This included 7 (19 %) cyclists who were conscious more than once daily. There was no significant difference in the frequency of weight consciousness between professionals and amateurs ($\chi^2_{1, 36} = 0.286$; $p = 0.60$) or between riders categorised based on best NRS race ranking ($\chi^2_{5, 36} = 5.96$; $p = 0.44$).

Body weight reduction techniques reported to have been practiced are reported in Figure 3. The most commonly selected techniques were avoiding foods high in fat ($n = 24$; 65%), avoiding foods high in sugar ($n = 23$; 62%), and reducing total daily energy intake (EI; $n = 28$; 76%). Other more extreme albeit less common practices were also reported; skipping meals ($n = 6$; 16%), training for prolonged periods without eating ($n = 7$; 19%), purging ($n = 2$; 5%), wearing additional clothing or plastic wraps while training ($n = 1$; 3%), and taking weight loss supplements or medications ($n = 1$; 3%).

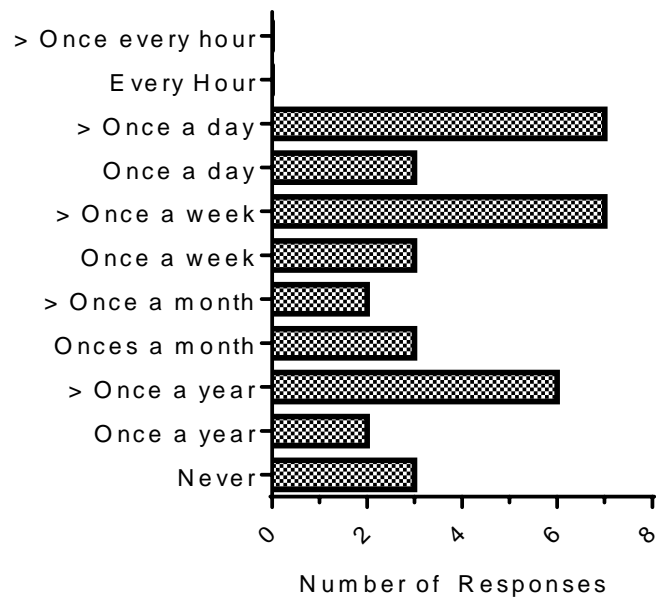


Figure 2. The frequency with which female cyclists report they were conscious about wanting to reduce their body weight in the past 12 months.

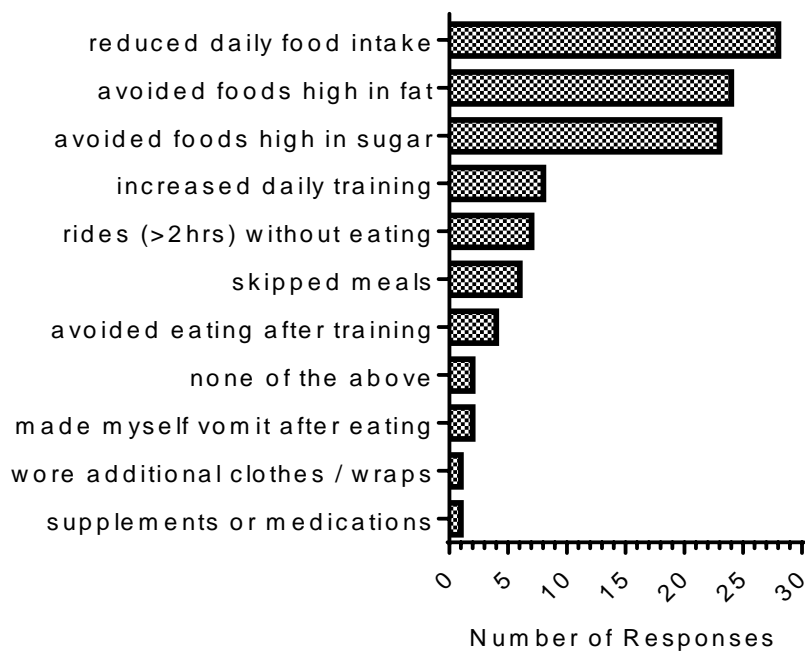


Figure 3. Self-reported techniques previously used for the specific purpose of reducing body weight.

3.3.4 Association between menstrual function and body weight

Of those cyclists who were not using a hormonal contraceptive ($n = 18$); 11 reported having had menstrual dysfunction in the past 12 mo. Ten reported that their menstrual cycle had ceased for more than 3 mo in the past year (amenorrheic). Of this sub-sample, those who reported menstrual dysfunction were more likely to be conscious about wanting to change their BW at least once weekly compared to those who were not ($X^2_{1,17} = 6.80$; $p = 0.02$). There were no main effects of menstrual function on the magnitude of BW change in the past 12 mo ($F_{1,10} = 0.872$; $p = 0.37$) or on current BW ($F_{1,16} = 1.21$; $p = 0.29$). When including all the cyclists regardless of hormonal contraceptive use, those who reported having an irregular menstrual cycle and/or going ≥ 3 mo without menstruating in the past 12 mo also reported a significantly lower current BW (-4.3 kg [0.7, 8.0]; $F_{1,35} = 5.78$; $p = 0.02$) and current BMI (-1.5 kg.m⁻² [0.1, 3.0]; $F_{1,34} = 4.69$; $p = 0.037$) compared to those who did not.

3.4 Discussion

The present study investigated weight consciousness, BW satisfaction in the context of performance, seasonal and career fluctuations in BW and BW manipulation in elite female cyclists. The primary finding from this investigation was that elite Australian female cyclists competing in major domestic races are a weight conscious population. A large proportion of the cyclists (54%) reported being conscious of wanting to change their BW at least once a week, with 19% being conscious of this desire more than once daily. These findings may suggest that a significant number of female cyclists have a longer term drive for thinness which may increase their risk of persistent energy deficiency.

It is important to note the subtle difference between these sport-related constructs and the constructs of social physique anxiety (Haase et al., 2002; Hart et al., 1989); body image concerns (Franzoi and Shields, 1984; Hausenblas and Downs, 2001; Varnes et al., 2013); eating disorders (Byrne and McLean, 2002; Sundgot-Borgen et al., 2013); eating attitudes (Byrne and McLean, 2002; Garner et al., 1982; Haase et al., 2002); and sociocultural pressure to be lean (Byrne and McLean, 2002; Reel et al., 2013), which have been reported on extensively in general and athletic populations. A unique construct investigated in this study is body weight satisfaction, which was framed specifically in the context of performance at a major competition. The majority of female cyclists (62%)

reported that their current BW was not ideal for performance in these races. They perceived that their ideal BW was on average (\pm SD) 1.6 ± 1.6 kg (2.5 ± 2.5 % body weight) less than the weight at which they presented at the event. Similarly, 67% of male cyclists in a study by Filaire et al. (2007) were dissatisfied with their current BW and the reported difference between current and preferred BW in other endurance athletes (runners, triathletes, orienteering, rowing swimming) was approximately 2.0 kg (Fogelholm and Hiilloskorpi, 1999). Previous research of female collegiate athletes has reported that those competing in higher division schools (Division 1 and II) experience greater body dissatisfaction, weight concern and preoccupation than those in Division III school athletic programs (Varnes et al., 2013). In this study, there were no significant differences in the magnitude of weight dissatisfaction related to performance between professional and amateur cyclists, nor was there an effect of best domestic race ranking.

While weight satisfaction was investigated in the context of performance at a specific competition, it may be that some of the findings reported persist throughout the season and are not race specific. This study measured weight consciousness and weight satisfaction at only one time point; leading into major competition. It is possible that perceptions towards body weight and its relation to performance could change throughout the season and possibly even following these races, depending on the outcome. Future studies should administer similar questionnaires throughout the year to investigate whether weight satisfaction can be isolated to a specific competition or whether it is a mindset that persists over longer time frames.

The level of dissatisfaction with BW at the Australian and Oceania Championships is interesting given that these races are preceded by the Australian Summer off-season where there has been sufficient training time and good environmental conditions to achieve desired body composition changes. It may be that for some athletes, there is a persistent drive for thinness regardless of the preparation made prior to major competition. It is possible that responses would be similar later in the season although this warrants further investigation particularly at larger international races e.g. World Championships or World Cups.

There was no significant difference between career lowest BW and the weight perceived to be ideal for these races. This suggests that a majority of these elite female cyclists not only wanted their current BW to be lower, but very close to their career lowest BW. This

supports the finding that 73% of the cyclists agreed that being at their career lowest BW was 'beneficial' or 'extremely beneficial' to race performance. It is also important to note that 7 (19%) of the cyclists would be classified as underweight ($BMI \leq 18.5 \text{ kg.m}^{-2}$) based on their estimated current BMI as well as their highest reported BMI (World Health Organization, 2000). The lowest BMI categorised 13 (35%) cyclists as underweight while the estimated ideal BMI placed 10 (27%) of the cyclists in the underweight category. It is concerning that a large number of female cyclists would be considered to have a BW that is unhealthily low relative to their height, by using World Health Organisation standards.

Unsurprisingly, the difference between current BW and ideal BW was non-significant for climbers whereas other types of cyclists had a significantly lower ideal BW for performance. Given the repetitive climbing on these courses and the fact that the current BW for climbers was lower than the other types of cyclists, this indicates that cyclists of different types may assess their BW against the course profile. With that said, the total vertical ascension of these two courses (~1500m) is still less than that experienced in isolated mountain passes frequently experienced in stage races such as the women's giro d'Italia or even domestic one day races such as the Tour of Bright. Furthermore, the winner of the recent National Championships would have been considered an all-rounder. Although the sample sizes were quite small for contrasts made between different types of cyclists, the power analysis revealed that with the magnitude of difference in body weight observed between climbers and the other types of cyclists, the study was sufficiently powered (89% probability of correctly rejecting the null hypothesis).

The secondary finding from this investigation was that a large proportion (~70%) of elite female cyclists competing at National and Oceania Championships reported attempts to manipulate their BW in the 12 mo prior to these races. The majority of these athletes were successful and reduced BW by an average of $2.4 \pm 1.0 \text{ kg}$ which represented $4.1 \pm 1.9\%$ of their current BW. This is similar to that reported for male cyclists ($3.1 \pm 0.3 \text{ kg}$) who reported using techniques such as fasting and laxatives (41%) and increased exercise volume (13%; Filaire et al., 2007). Other reviews of weight loss practices of elite male cyclists have noted substantial restriction of EI in general or at certain times of the day (e.g. evening), undertaking training rides in an overnight fasted state, use of anorexic drugs and undertaking high volume stage races without increasing EI (Burke, 2001). In the present female cyclists, reductions in BW were primarily brought about by reducing daily EI, avoiding foods high in fat, avoiding foods high in sugar and increasing training

load. Of greater concern was the small number of cyclists who performed what may be described as more extreme weight loss practices, such as skipping meals, training for prolonged periods without eating, purging, wearing additional clothing or plastic wraps while training, and taking weight loss supplements or medications. It should be emphasised that this represented only a small number of the cyclists surveyed (see Figure 3). Nonetheless, there is sufficient evidence to suggest that female cyclists not engaging in extreme weight loss practices may be attempting to reduce EI relative to exercise energy expenditure (EE), thereby placing themselves at risk of a persistent energy deficiency and its associated health and performance risks.

It is noteworthy that in a group of 37 female cyclists, 5 (14%) had been previously diagnosed with an eating disorder by a physician and 12 (32%) reported that their coach or sports director had been dissatisfied with their body weight in the past 12 mo. While BW and indeed body composition have performance implications on cycling performance, consideration must be given to the way this message is delivered. It is acknowledged that the actual prevalence of eating disorders and disordered eating may be higher in this group and the absence of a validated questionnaire assessing these constructs more thoroughly (e.g. Eating Disorder Inventory: Garner et al., 1983, or The Eating Disorder Examination: Cooper and Fairburn, 1987) is a limitation of the study. For instance, Riebl et al. (2007) previously reported that from a group of 61 male cyclists, 12 provided responses consistent with disordered eating whereas only 5 self-reported having an eating disorder. Similar results could be expected from female cyclists. The absence of such a questionnaire makes it difficult to distinguish weight conscious athletes from those at risk of chronic or excessive energy deficiency and/or behaviours consistent with disordered eating.

Williams et al. (2006) reported that irregular menstrual cycle length was modestly associated with a higher risk of disordered eating in female athletes. Furthermore, it is well documented that low energy availability impacts negatively on menstrual function (Loucks et al., 2011). Melin et al. (2014) have shown that self-reported menstrual dysfunction has a high specificity and sensitivity for clinically verified menstrual dysfunction. Therefore, the FCWM Questionnaire included a series of questions designed to investigate the association between menstrual cycle regularity, magnitude of BW change and current BW. This study was limited in sample size and the questionnaire failed to ask why the cyclists used a hormonal contraceptive; it is not uncommon that

females with hypothalamic amenorrhea are prescribed a hormonal contraceptive in order to improve or maintain bone mineral density. These limitations notwithstanding, there was evidence that female cyclists with menstrual dysfunction had a lower current BW and were more frequently conscious about wanting to change their BW. Furthermore, of those athletes who were not using hormonal contraceptives, 61% reported having menstrual dysfunction. These combined findings, suggest a large number of these cyclists have a more persistent suppression of endocrine function which may be related to energy deficiency.

It is widely accepted that functional hypothalamic menstrual disorders as well as low bone mineral density stem from energy deficiency (Loucks et al., 2011; Mountjoy et al., 2014; Nattiv et al., 2007; Sangenis; Sherman and Thompson, 2006; De Souza et al., 2014). One of the more serious health implications of energy deficiency is low bone density. While this study did not measure bone density, it did reveal evidence of risk factors for energy deficiency in female cyclists. Female cyclists have been reported to have a high prevalence of poor bone health (Sherk et al., 2014) possibly owing to a combination of persistent energy deficiency and low mechanical bone loading (Schofield and Hecht, 2012). Future studies should investigate relationships between energy deficiency and bone health in this population as osteoporosis is one of the more debilitating outcomes associated with prolonged energy deficiency.

3.4.1 Conclusion

These results suggest that Australian female cyclists are a weight conscious population who are frequently motivated to reduce BW and consciously do so seasonally. It is of concern that the majority of respondents were dissatisfied with their BW leading into a major competition. This, coupled with the relatively high incidence of previous eating disorder diagnoses, an apparent drive to be underweight ($BMI \leq 18.5 \text{ kg/m}^2$) and the prevalence of menstrual dysfunction supports the opinion that this athletic population is at risk of engaging in practices which may result in deliberate or inadvertent energy deficiency relative to EI. This may place them at greater risk of compromising their health and performance. While weight dissatisfaction was affected by the type of cyclist (or perhaps by the course profile) this investigation did not show that professional status or domestic race results influenced this. There was evidence that BW reducing practices and weight consciousness may be associated with menstrual dysfunction although this study

did not seek to establish causality. Given that road cycling is a weight sensitive sport, it is perhaps not surprising that athletes participating in the sport manipulate their BW and perceive benefit in a low body weight. The high incidence of weight dissatisfaction is, however, noteworthy for support staff engaging with these athletes. These results underscore the importance for coaches, sports directors and sports scientists to be sensitive and provide appropriate instruction and ongoing support when advising athletes on exercise and diet strategies that may affect energy availability and body composition. In a population that is weight conscious and motivated to have a low BW, strategies aimed at achieving this should place primary emphasis on maintaining good health.

CHAPTER 4: BODY COMPOSITION IN WORLD CLASS, ELITE AND SUB-ELITE FEMALE ROAD AND TRACK ENDURANCE CYCLISTS: NORMATIVE VALUES AND TYPICAL CHANGES

4.1 Introduction

There are two main resistive forces that influence the energy cost of cycling: air resistance when cycling on flat roads and gravity when cycling uphill. An increase in body weight (BW) increases the energy requirement to overcome the resistive forces of gravity when climbing. Models of cycling performance have suggested that a 1 kg increase in BW can increase cycling time up a 5% grade by ~1% (Olds et al., 1995) which in international competition is significant. Impellizzeri et al. (2008) have shown that female time trialists and flat-land specialists have a higher BW, body surface area (BSA) and frontal area (FA) than climbers and mountain bikers. The maximal aerobic power (MAP) of these cyclists in absolute terms and relative to these morphological characteristics does not appear to scale in direct proportion to BW. Impellizzeri et al. (2008) and Swain et al. (1987, 1994) have described the scaling of power output (W) to BW (kg) in cyclists as allometric ($W/kg^{0.32}$ on flat roads; $W/kg^{0.79}$ on climbs) showing that specialisation (climber, time trialist, flat-land) in elite cycling is in part morphology dependent. While an athlete's morphology is somewhat predetermined, body composition can certainly be manipulated (Haakonssen et al., 2013). By reducing non-functional mass and optimising functional lean muscle and thus power output in relative terms, cyclists have the potential to improve their performance. It is also noteworthy that women's road cycling is characterised by many accelerations (Ebert et al., 2005) and that acceleration is inversely proportional to weight. Because of these power-weight relationships, it is not uncommon that athletes and coaches use techniques that allow cyclists to manipulate BW to improve the power-to-weight ratio and thus climbing and sprint performance.

Martin et al. (2001) have provided the most recent data on the anthropometry and physiological variables of female road cyclists, finding that the internationally competitive cyclists ($n = 4$) were lighter and leaner than their lesser successful team mates. This study was cross-sectional in analysis, is ~13 y old and the sample size was small ($n = 12$). Research is yet to describe the body composition of a large sample of competitive female road and track cyclists and its association with performance level.

Anthropometry is useful for describing fat mass using skinfolds (Norton and Olds, 1996). More recently, techniques have been developed to describe lean mass changes using anthropometry. The use of lean mass index (LMI) was originally proposed by Slater et al. (2006) as a method to track changes in weight independent of changes in fat mass and is therefore a metric of lean mass changes. Lean mass index is calculated as BW/S^x where 'BW' is body weight; 'S' is sum of seven skinfolds and 'x' is an exponent based on the slope of the relationship between BW and skinfolds in the studied population. While this exponent has been determined for rugby players (Duthie et al., 2006) and male and female swimmers (Pyne et al., 2006), it has not been customised for female cyclists and LMI has not been described for females cyclists. Similarly, seasonal and career variations in body composition of female cyclists have not been reported. Better describing normative values and typical changes in the body composition of female cyclists will better guide coaches and athletes as to what can realistically be achieved.

The primary purpose of this study was to describe normative values for anthropometry in a large sample of Australian world class, elite and sub-elite female road track and endurance cyclists over 16 y and to investigate the differences based on these classifications. It was hypothesised that higher performing athletes would have lower BW and skinfolds than those performing at a lower level. The secondary purpose was to describe seasonal variations in anthropometry observed in this population. It was hypothesised that athletes would be lighter and leaner during the competition months compared to the off-season. The final purpose of this study was to validate the use of LMI and describe this measure in female cyclists.

4.2 Methods

This study had institutional ethics approval from the University of Queensland and the Australian Institute of Sport (AIS) and all subjects provided written informed consent prior to participating. Australian female cyclists (n = 126) involved with the AIS through the National High Performance Program provided anthropometric data over a period of 16 y (1998 – 2014). Subjects included developmental athletes from State Sports Institutes and National Road Series teams, AIS / Cycling Australia scholarship holders and professional cyclists who came through the National Program or returned to represent the National Team at World Championships, Commonwealth Games and Olympic Games. All anthropometric data were collected by International Society for the Advancement of

Kinanthropometry (ISAK) accredited anthropometrists (n = 7), with a relative technical error of measurement (TEM) between 1.5 - 2.5% based on repeated measurements on 20 cyclists. All subjects presented for testing in the morning in a fasted state. Body weight was recorded to the nearest 0.01 kg using a calibrated scale (A&D HW-200KGL Sydney, Australia) with cyclists wearing only light underwear. Skinfold thickness (mm) was measured to the nearest 0.1 mm in duplicate (triplicate when the second measure was $\geq 5\%$ different to the first at which point the median value was used) using calibrated calipers (Harpenden West Sussex, England) at seven sites using techniques previously described by Stewart et al. (2011). The skinfold sites used were triceps, subscapular, biceps, supraspinale, abdominal, front thigh and medial calf. Percentage body fat (BF%) was estimated using Equation 4 (Siri, 1961) which relies on body density (BD) estimates calculated using Equation 5 (Withers et al., 1987b). Body surface area was estimated using Equation 6, described by Du Bois and Du Bois (1989), and frontal area (FA) was calculated using Equation 7 (Padilla et al., 1999).

Equation 4

$$\text{BF\%} = (4.95 / \text{BD}) - 4.5) \times 100$$

Equation 5

$$\text{BD} = 1.17484 - 0.07229 \times \log_{10}(\text{triceps} + \text{subscapular} + \text{supraspinale} + \text{calf skinfolds})$$

Equation 6

$$\text{BSA} = 0.007184 \times \text{BW}^{0.425} \times \text{height}^{0.725}$$

Equation 7

$$\text{FA} = 0.185 \times \text{BSA}$$

4.2.1 Effects of performance level and season phase on anthropometry

Cyclists were classified as world class, elite, or sub-elite according to their performances during the year corresponding to their anthropometric profile; criteria used for classification are presented in Table 7. The date when each profile was measured was categorised to a season phase: off-season (October 1 – December 31); early-season (January 1 – April 30); and late-season (May 1 – September 30). It should be noted that formal training is performed during the off-season, although the volume and intensity as well as the number of races performed would be expected to differ significantly compared to in-season. Athletes classified as Junior (18 - 19 y) were removed from the data set. For road cyclists, anthropometric and morphological variables were investigated using linear mixed-effects modelling fit by restricted maximum likelihood estimation (REML) with performance level (sub-elite, elite, world class) and season phase (off-season, early-season, late-season) as fixed effect and subject as a random effect. Non-significant terms were dropped from the model. A subsequent model was created using month of the year as a fixed effect. There was too few track endurance cyclists with profiles measured at the lower levels to make meaningful inferences. Tukey's HSD post-hoc analysis was used to identify the differences. A Student's T-test was used to compare measures corresponding with career lowest weights for world class cyclists categorised as road and track endurance.

Table 7. Performance level of cyclists based on race results, international ranking and team selection

<i>Sub-elite</i>	<i>Elite</i>	<i>World class</i>
<ul style="list-style-type: none"> • National Road Series registered athlete • State Sport Institute Scholarship holder • Talent identification / transfer athlete 	<ul style="list-style-type: none"> • National team representative • Australian Institute of Sport / Cycling Australia scholarship holder • Racing professionally in Europe USA • Senior National Championship road race, time trial or track endurance* medallist 	<ul style="list-style-type: none"> • UCI[†], World Cup or CQ^{††} top 10 ranking • UCI 1.1 race winner • UCI 2.1 race top 3 and/or stage winner • UCI World Cup road or track endurance* medallist • UCI World Championship Road Race, Individual or Team Time Trial medallist • Olympic Games road race, time trial or track endurance* medallist

*Track endurance events include: individual pursuit; team pursuit, omnium and scratch race

[†]Union Cycliste Internationale (UCI) ranking system (Vanderwegen et al., 2014)

^{††}Cycling Quotient (CQ) ranking is a non-official ranking system based on world performances of professional cyclists in the previous 12 months which is at times considered more accurate than the

4.2.2 Lean mass index

Lean mass index was calculated as BW / S^x (Slater et al., 2006). The exponent (x) was calculated as the slope of the natural logarithm of BW and skinfolds. To establish a population specific exponent, the slope was calculated for each cyclist who had 6 or more anthropometric profiles all measured by the same anthropometrist while over the age of 19 y. The mean of these individual exponents was then used to calculate LMI for each athlete's profile. In a sub-set of cyclists, percentage changes in LMI were compared to percentage changes in lean mass measured using dual-energy X-ray absorptiometry (DXA; narrowed fan-beam; Lunar Prodigy; GE Healthcare, Madison, WI). DXA scans were performed on the same morning of the anthropometric profile, in a fasted state using standardised techniques developed by Nana et al. (Nana et al., 2012a). Pearson's correlation coefficients were used to explain the variance of DXA delta lean mass (%)

explained by delta LMI (%). The frequency with which LMI identified the correct direction of change in DXA lean mass was also calculated.

A difference of 1.00 kg BW was deemed clinically significant where this could contribute to a 1 % change in performance (Olds et al., 1995) and would most likely exceed biological variability. A difference of 5.0 mm skinfolds was deemed clinically significant as this was found to be the average slope (5.0 mm / 1 kg BW) of the linear relationship between skinfolds and BW in a subset of cyclists who had >10 profiles. Based on a reference female cyclist (BW: 58.5 kg; skinfolds: 63.0 mm), a LMI difference of 0.5 was deemed clinically significant as this approximated the LMI change corresponding to a 1 kg difference in BW or a 5 mm difference in skinfolds independent of a change in skinfolds or BW respectively. An alpha of 0.05 was assumed for statistical analyses. Statistical analyses were conducted using JMP Pro® v10 (SAS Institute Inc., Cary, NC, USA). Unless otherwise stated, results are written as means and 95 % confidence intervals (\bar{X} [95% CI]).

4.3 Results

A total of 616 anthropometric profiles were recorded from 126 female cyclists. Of the 91 cyclists who had more than one profile recorded, the average duration over which they were monitored was 2.49 y [2.15, 2.96] with a median of 2.0 and a maximum duration of 9.57 y. Results for the association between anthropometric and morphological variables and performance level are shown in Table 8.

Table 8. Descriptive statistics for anthropometry of sub-elite, elite and world class female road cyclists

	<i>Sub-elite</i>	<i>Elite</i>	<i>World class</i>
	\bar{X} [95% CI]	\bar{X} [95% CI]	\bar{X} [95% CI]
<i>Age</i> (y)	25.6 ^{E, W} [24.8, 26.4]	25.6 ^{S, W} [23.1, 24.7]	27.0 ^{S, E} [26.1, 27.8]
<i>Height</i> (cm)	169.2 ^W [168.1, 170.4]	169.3 ^W [168.1, 170.4]	170.0 ^{S, E} [169.0, 171.4]
<i>Body Weight</i> (kg)	60.71 ^W [59.68, 61.74]	60.14 ^W [59.11, 61.16]	59.54 ^{S, E} [58.46, 60.61]
<i>Skinfolds</i> (mm)	70.5 ^W [67.3, 73.8]	67.8 ^W [64.6, 71.0]	63.1 ^{S, E} [59.6, 66.7]
<i>BF</i> (%)	16.0 ^W [15.3, 16.1]	15.5 ^W [14.8, 16.1]	14.5 ^{S, E} [13.7, 15.2]
<i>LMI</i> (kg / mm ^{0.15})	31.91 [31.4, 32.38]	31.80 [31.33, 32.27]	31.84 [31.36, 32.33]
<i>BSA</i> (m ²)	1.696 [1.677, 1.715]	1.690 [1.671, 1.708]	1.689 [1.669, 1.708]
<i>FA</i> (m ²)	0.314 [0.310, 0.317]	0.313 [0.309, 0.316]	0.312 [0.308, 0.316]
<i>Subjects</i> (Measures)	76 (142)	60 (333)	32 (141)

Results presented as means (\bar{X}) and 95% confidence intervals (CI)
Body fat (BF); lean mass index (LMI); body surface area (BSA); frontal area (FA).

Significantly different ($p < 0.05$) to sub-elite^S; elite^E; world class^W

4.3.1 Normative values and seasonal changes in anthropometry

There were no statistically significant interactions between performance level and season phase for any of the variables. Compared to sub-elite and elite female road cyclists, world class road cyclists were on average 2.8 y [2.2, 3.5] and 1.1 y [0.6, 1.6] older; weighed 1.18 kg [0.46, 1.90] and 0.60 kg [0.05, 1.15] less; and had skinfolds that were 7.4 mm [3.8, 11.0] and 4.6 mm [1.8, 7.4] lower respectively. There were no statistically significant ($p = 0.054$) differences between sub-elite and elite cyclists although average BW (0.58 kg [-0.01, 1.16]); skinfolds (2.8 mm [-0.2, 5.7]) and BF% (0.5 % [-0.1, 1.1]) were lower in elite cyclists. There were main effects for season phase (see Table 9) with higher values measured for BW (0.41 kg [0.04, 0.77]); skinfolds (4.0 mm [2.1, 6.0]); BF% (0.9 % [0.5, 1.3]); BSA (0.006 m² [0.0001, 0.013]) and FA (0.002 m² [0.0001, 0.003]) in the off-season compared to the early-season. While there was no significant effect of Month for BW ($F_{11, 487} = 1.56$; $p = 0.11$) the highest average BW was in December (60.66 kg [59.62, 61.70]) and the lowest in February (59.88 kg [58.84, 60.93]). There were significant effects of Month for skinfolds ($F_{11, 496} = 4.04$; $p < 0.001$). Skinfolds were higher in December (70.6 mm [67.2, 73.9]) and November (71.2 mm [67.6, 74.7]) compared to February (65 mm [62.1, 68.9]), April (64.5 mm [60.1, 68.8]), August (63.9 mm [59.4, 68.5]) and September (65.3 mm [61.3, 69.3]).

4.3.2 Comparison between world class road and track endurance cyclists

Compared to track endurance cyclists at the world class level, female road cyclists were statistically significantly older (4.4 y [1.4, 7.4]); had lower BW (6.04 kg [2.73, 9.35]); skinfolds (11.5 mm [1.1, 21.9]) and BF % (2.7 % [0.2, 5.2]). Lean mass index (2.14 kg / mm^{0.15} [0.53, 3.75]); BSA (0.090 m² [0.009, 0.170]) and FA (0.017 m² [0.001, 0.033]) were higher in track endurance cyclists compared to road cyclists (see Table 10). On average, track endurance cyclists were taller than road cyclists although this was not statistically significant (5.2 cm [-0.5, 10.9]).

Table 9. Descriptive statistics for anthropometric measures at different points of the season

	<i>Off-season</i> \bar{X} [95% CI]	<i>Early-season</i> \bar{X} [95% CI]	<i>Late-season</i> \bar{X} [95% CI]
<i>Weight</i> (kg)	60.37 ^E [59.35, 61.39]	59.97 ^O [58.95, 60.98]	60.04 [59.03, 61.06]
<i>Skinfolds</i> (mm)	69.6 ^{E, L} [66.5, 72.8]	65.6 ^O [62.5, 68.7]	66.2 ^O [63.1, 69.3]
<i>BF</i> (%)	15.9 ^{E, L} [15.3, 16.5]	15.0 ^O [14.3, 15.6]	15.0 ^O [14.4, 15.7]
<i>LMI</i> (kg / mm ^{0.15})	31.79 [31.32, 32.26]	31.87 [31.40, 32.33]	31.90 [31.43, 32.36]
<i>BSA</i> (m ²)	1.695 ^{E, L} [1.676, 1.714]	1.688 ^O [1.670, 1.707]	1.691 [1.672, 1.709]
<i>FA</i> (m ²)	0.314 ^{E, L} [0.310, 0.317]	0.312 ^O [0.309, 0.316]	0.312 [0.309, 0.316]
<i>Number of</i> <i>Subjects</i> (Measures)	94 (177)	81 (245)	80 (194)

Results presented as means (\bar{X}) and 95% confidence intervals (CI).

The date when each profile was measured was categorised to a season phase; *off-season* (October 1 – December 31); *early-season* (January 1 – April 30) and *late-season* (May 1 – September 30).

Body fat (BF); lean mass index (LMI); body surface area (BSA); frontal area (FA). Significantly different ($p < 0.05$) to *off-season*^O; *early-season*^E; *late-season*^L.

Table 10. Anthropometric measures corresponding with the lowest body mass for world class road cyclists and track endurance cyclists

	<i>Road cyclists</i>	<i>Track endurance cyclists</i>
	\bar{X} [95% CI]	\bar{X} [95% CI]
<i>Age</i> (y)	26.6 [24.8, 28.3]	22.2 * [19.7, 24.6]
<i>Height</i> (cm)	168.7 [166.0, 171.3]	173.9 [168.9, 178.9]
<i>Weight</i> (kg)	57.12 [55.18, 69.06]	63.16 * [60.48, 65.84]
<i>Skinfolds</i> (mm)	51.9 [45.8, 58.0]	63.4 [55.0, 71.9]
<i>BF</i> (%)	11.8 [10.4, 13.3]	14.6 * [12.6, 16.6]
<i>LMI</i> (kg / mm ^{0.15})	31.50 [30.56, 32.45]	33.64 * [32.34, 34.95]
<i>BSA</i> (m ²)	1.650 [1.612, 1.689]	1.740* [1.669, 1.811]
<i>FA</i> (m ²)	0.305 [0.297, 0.312]	0.312* [0.308, 0.336]
<i>Subjects</i>	21	11

Results are presented as means (\bar{X}) and 95% confidence intervals (CI). Track endurance events include the individual pursuit; team pursuit, omnium and scratch race.

Body fat (BF); lean mass index (LMI); body surface area (BSA); frontal area (FA).

*Significant difference ($p < 0.05$).

4.3.3 Lean mass index

There were 32 athletes for whom LMI was calculated. The average exponent for LMI used for the rest of the sample was 0.15 [0.13 – 0.18]. LMI explained 87% of the variance in DXA lean mass ($p < 0.001$; see Figure 4). There were 51 comparable changes scores for LMI and DXA lean mass from 25 athletes (mean [90% CI]; 2.0 [1.4 – 2.7]). Percentage LMI change explained 44% of the variance in percentage DXA lean mass change ($p < 0.001$; see Figure 5). LMI accurately predicted the direction of change in DXA lean mass in 65 % of cases. When the change in DXA delta lean mass was $\geq 1\%$, LMI predicted the direction of change in 76% of cases.

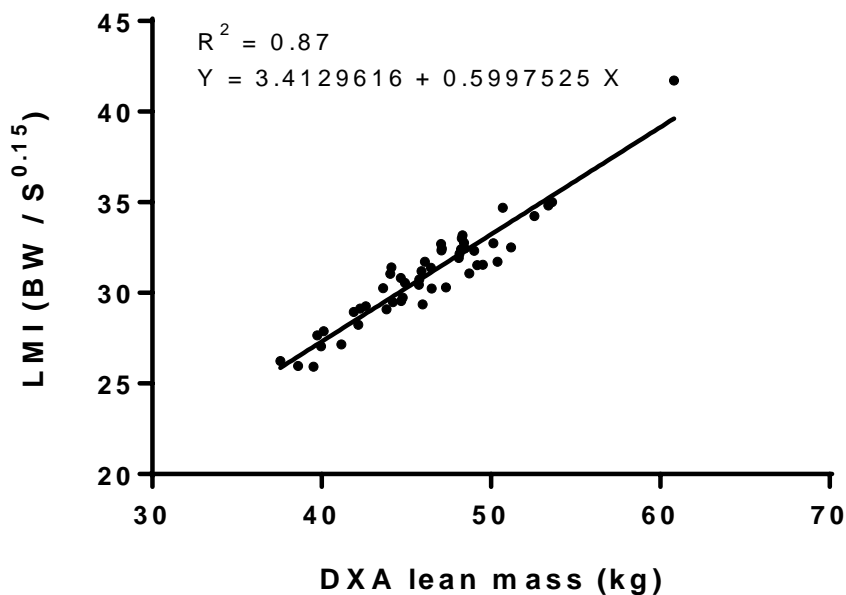


Figure 4. The relationship between lean mass index (LMI) and lean mass measured using dual energy x-ray absorptiometry (DXA) in female cyclists. LMI is calculated as body weight (BW) divided by skinfolds (S) raised to a population specific exponent. For female cyclists, the exponent is estimated to be 0.15.

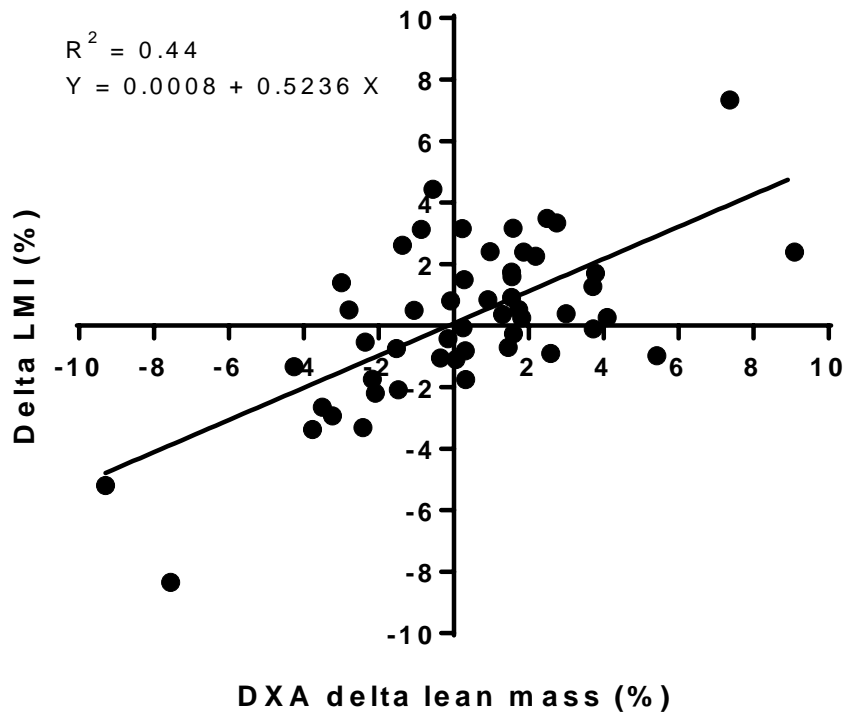


Figure 5. The relationship between changes in lean mass measured using lean mass index (LMI) and changes measured using dual energy x-ray absorptiometry (DXA). Both delta scores are represented as a percentage of the baseline measure.

4.4 Discussion

Body composition is a trait of female cyclists that is believed to have an impact on performance (Haakonssen et al., 2014a). A key performance indicator for both male and female cyclists is the maximal amount of power that can be generated for a given duration relative to BW. The purpose of the present study was to provide and assess contemporary normative data for BW and skinfolds in clearly defined groups of sub-elite, elite, and world class female Australian cyclists. World class female road cyclists weigh less (~1.0 kg) and have lower skinfolds (~5.0 mm) and estimated BF% (~1.5 %) compared to road cyclists performing at lower levels. The differences between cyclists of different performance levels in this study are similar in magnitude to those reported previously (Martin et al., 2001).

In order to better describe the World class cyclists when they were theoretically well trained, the anthropometric profiles corresponding with their lowest BW on record were presented. At their lowest recorded BW, the average BW (~57 kg) and skinfolds (~52 mm)

for World class female road cyclists were comparable to those reported by Martin et al. (2001), although the spread in this study was greater than previously reported. Body weight ranged from 48.0 – 65.7 kg and skinfolds from 31.1 – 85.2 mm. In road cycling, the physiology and morphology of cyclists can dictate that they specialise as sprinters, time trialists or climbers. Compared to other endurance sports, this makes the population somewhat heterogeneous in terms of anthropometry and morphology. The larger sample of athletes in this study, likely accounts for the greater variability in anthropometric measures compared to earlier studies. It is also noteworthy that the average BW and estimated BF% was lower than early studies investigating anthropometry in female cyclists (Burke, 1980; Pfeiffer et al., 1993; Wilber et al., 1997). This suggests that today's female road cyclist may engage in training or dietary practices that are different to those of their predecessors.

Due to typical changes in training and racing volume throughout the year, it was hypothesised that BW and skinfolds would oscillate also. Body weight and skinfolds were higher in the off-season compared to in-season, leading to higher estimates of FA and BSA at this time also. This may have implications not just for an athlete's relative power output (W/kg) but also their aerodynamic drag. Despite main effects for performance level and season point, there was no interaction between these variables. Likewise, there was no interaction between performance level and month of the year which accounts for seasonal differences that might occur particularly between sub-elite cyclists who race domestically and the elite and world class cyclists who follow a slightly different race schedule internationally. This may be due to an insufficient frequency of monitoring across all months.

Although anthropometric monitoring has been conducted at the Australian Institute of Sport for over 16 y, the frequency of monitoring individual athletes was not as great as that which has been reported for sports such as swimming (Anderson et al., 2006). A higher frequency of monitoring allows the sport scientist to make more meaningful inferences by relating anthropometry to training phases and performance, as well as describing changes that occur from season to season. In distance based sports such as swimming and running, training and competition can be relatively standardised so accurate and reliable performance measures are somewhat more accessible than for road cycling, which is characterised by many oscillations in speed due to the highly strategic nature of the sport. Nonetheless, standardised testing protocols utilising power output for specific durations

can certainly be used in training and the frequency of anthropometry increased to better understand how it relates to performance.

The anthropometric values for world class road cyclists were substantially lower than for world class track endurance cyclists who were on average ~6 kg heavier and had skinfolds ~11 mm greater. The LMI of track endurance cyclists was substantially higher than for the road cyclists. Higher power output demands associated with track endurance events such as the individual and team pursuit may necessitate greater functional lean mass particularly at the world class level. Absolute power may be more important for performance on the track compared to relative power (W/kg), given that track endurance cyclists do not have to overcome gravity to the same extent that as road cyclists and the only significant acceleration occurs during the start. It is also noteworthy, that the average age for track endurance cyclists was significantly younger than for the road cyclists. This is not surprising given that many junior athletes start on the track and then progress to the road. Also, Australia has a history of female road cyclists entering the sport after adolescence, sometimes through talent transfer from other sports such as rowing or running. While there was no correlation between age and skinfolds, it may still be that age or maturity has an influence on leanness (skinfolds) more so than discipline (road or track endurance). Younger athletes participating in track disciplines may not have developed their dietary knowledge and practical skills to achieve body composition measures similar to those of older road cyclists. This is purely speculative and requires further investigation.

Recently, imaging technologies such as DXA have been become more accessible to athletes and sport scientists. Dual energy X-ray absorptiometry provides a useful tool for occasional monitoring of athletes; for accurately determining energy intake (EI) requirements based on lean mass, or for validation of other anthropometric based measures. In the daily training environment, anthropometry still prevails as the most practical, routine measure of body composition in athletes. The use of a validated, field based method to estimate changes in lean mass could be useful to better describe body composition changes in female cyclists without the need for less practical methods such as DXA.

Martin et al. (2001) have previously differentiated between levels of cyclists by internationally competitive and nationally competitive, while Impellizzeri et al. (2008) classified female cyclists as world class if they obtained a top 15 place in a World Cup,

World Championship, or international stage race competition. Given the nature of cycling, whereby some athletes sacrifice their own performance in aid of a team leader, the use of results alone is not sufficient to differentiate between cyclists as sub-elite, elite and world class. It is for this reason that this study used more extensive criteria for these categories.

This study has a number of limitations. Seven different anthropometrists recorded profiles at various times over the duration of data collection. While this may have led to inter-tester error, this was minimised by standardised measurement procedures and training. There was also a sample bias within the different groups. The majority of profiles were taken when cyclists were at the elite level and although bias was minimised by using only minimum values, it is more likely that the true minimum of the year was recorded for elite cyclists compared to world class and sub-elite cyclists who had a lower frequency of monitoring. Future studies should monitor well defined groups of cyclists throughout the same season, at the same time points and with a similar frequency. While this study has begun to show differences between and within cyclists, a more comprehensive study is required to elucidate the magnitude of seasonal variation that could be expected and the time course of such changes. This will help develop reasonable targets for strategies used to manipulate body composition. Work is also required to determine the biological variability in BW and skinfolds in this population which may be affected by fluid shifts throughout the menstrual cycle.

Population prediction equations are often applied to anthropometric data to estimate BD and from this FM% (Withers et al., 1987a, 1987b, 1987c). Equations for BD use equations to estimate BF% (Siri, 1961) or (Brožek et al., 1963) that make the following assumptions: there is constant compressibility of skin and subcutaneous fat; there are no differences in individual fat distribution patterns; skinfold thickness is directly proportional to total body fat mass; the density of fat mass and fat free mass are 0.900 and 1.100 g/cm³ respectively; the proportion of the components of fat free mass (water, protein, bone mineral and non-bone mineral) do not vary between individuals; and the densities of the components of fat free mass do not vary between individuals (Norton and Olds, 1996). Skinfolds are certainly a practical tool and generalisations can be made about population specific anthropometry, although the afore mentioned limitations should be considered. While estimates for fat mass can be calculated, often comparing raw skinfold results to reference data or to an individual's previous measures is more meaningful. Caution must be taken particularly when comparing results between individuals and further caution must be taken

when comparing results for fat mass to other body composition measurement techniques e.g. DXA, bioelectric impedance analysis.

4.4.1 Conclusion

A high power-to-weight ratio is an important physiological characteristic for cyclists to be able to climb and accelerate quickly. This study provides evidence that there is a relationship between performance and body composition in female cyclists. World class Australian female cyclists have lower BW and skinfolds compared to elite and sub-elite female cyclists. This study provides contemporary normative data which can be referenced for female cyclists at various levels in their career. The findings may help guide coaches, sports scientists and support staff to understand what is reasonably required to achieve at a world class level. The use of LMI appears to be a valid monitoring tool for lean mass changes although this technique has a low sensitivity for changes in lean mass less than one kilogram. Future studies should use a high frequency (>monthly) of monitoring to better elucidate seasonal variation in body composition.

CHAPTER 5: LEAN MASS - CYCLING POWER RELATIONSHIPS IN FEMALE ROAD CYCLISTS

5.1 Introduction

Road cycling races are characterised by variable power output produced over long durations (>4 h). These demands require athletes to possess both high aerobic and anaerobic capacities in addition to functional muscle mass to produce the necessary mechanical work. Sprinting and climbing performance are influenced by both total body weight (BW) and power output. Because of this, road cyclists often attempt to improve power output relative to BW by reducing total BW. What is less frequently considered is the preservation of lean mass with high functional value. Researchers and sport scientists typically describe cyclists' physiology by their aerobic capacity, using expired gas analysis, lactate kinetics (Martin et al., 2001; Mujika and Padilla, 2001) or inferred through sustained power outputs >1 min (Quod et al., 2010) or anaerobic capacity, for example power output over short durations <1 min (Quod et al., 2010), anaerobic work capacity (Bergstrom et al., 2012; Skiba et al., 2014) or maximal accumulated oxygen deficit (Noordhof et al., 2010). With regards to body composition, the emphasis is typically placed on total BW and body fat percentage (Impellizzeri et al., 2008; Martin et al., 2001; Mujika and Padilla, 2001) given that optimising power relative to BW has implications for climbing and accelerating in sprints. While maintaining a low BW is certainly important, maintaining sufficient functional lean mass to maintain the power output demands of competition is also important. The power output of cyclists has been described in absolute terms (W) and relative to BW (W/kg) in the context of performance demands of women's (Ebert et al., 2005; Impellizzeri et al., 2008) and men's (Ebert et al., 2006; Quod et al., 2010) professional races, as well as laboratory testing of males (Lee et al., 2002; Mujika and Padilla, 2001; Quod et al., 2010) and females (Impellizzeri et al., 2008; Martin et al., 2001). However, little attention has been paid to the functional muscle requirements of elite road cyclists, particularly females.

Martin et al. (2000) reported that thigh muscle volume and optimal pedalling rate (cadence corresponding with peak power output) explained ~83% of the variance in instantaneous maximum power output during sprint cycling in males aged 8 – 70 y. These findings are supported by previous research validating the use of MRI for an accurate measure of muscle mass (Mitsiopoulos et al., 1998) and that optimal pedalling rate is closely related to

vastus lateralis muscle fibre type (Hautier et al., 1996). Establishing relationships between maximal power output and lean mass could be used to better describe the muscle quality of elite cyclists and to guide body composition manipulation strategies and strength and power training methods. By considering performance relative to functional mass (lower body lean mass) and non-functional mass (body fat, upper body lean mass) as opposed to just total BW, training and diet interventions could be better constructed to optimise performance outcomes.

While previous work has provided clear evidence of a relationship between muscle volume and instantaneous maximum power output, less attention has focused on the contribution of functional mass to maximal cycling efforts of longer duration which are more reflective of the demands of road cycling. Longer efforts will certainly be influenced by supporting physiological characteristics such as heart size, total haemoglobin mass, muscle capillary and mitochondrial density, and aerobic enzyme activity. Nonetheless, it is likely that functional mass contributes significantly, not only to sprint performance but also to efforts of longer durations. By quantifying normative relationships between power and functional mass, cyclists can be better characterised and training interventions can become evidence based. Therefore, the purpose of this study was to describe the contributions of lower body lean mass towards maximal power output over efforts of increasing duration in elite female cyclists.

5.2 Methods

Amateur female road cyclists were recruited through State Sports Institutes and Academies and National Road Series teams to take part in Australian National Women's Road Program selection camps. During these camps, a number of laboratory based performance tests were conducted in the first three days to determine whether cyclists would progress in the selection process. This study was approved by the Human Research Ethics Committee of the Australian Institute of Sport. Subjects were informed of testing protocols and risks of the study before providing written informed consent. Cyclists completed a Power Profile (PP; Quod et al., 2010) on a wind braked ergometer (Wattbike Ltd, Nottingham, UK). The PP test protocol consisted of seven maximal efforts (6, 6, 15, 30, 60, 240 and 600 s) with active recovery periods of 54, 74, 225, 330, 480 and 600 s respectively. The ergometer had been calibrated as previously described (Abbiss et al., 2009; Maxwell et al., 1998; Woods et al., 1994) using a custom built first principles

dynamic calibration rig (Tom Stanef, SASI, Australia). Following calibration, the mean (\pm SD) error was $0.7 \pm 0.5\%$ across a range of cadences (50 - 120 rpm) and power outputs (80 - 900 W) representative of road cycling. Maximal Mean Power (MMP) was recorded for each bout and the highest instantaneous 1 s and 5 s MMP were recorded from the 6 s efforts. During the 15 s and the 30 s efforts, the tester gently adjusted the resistance to keep the cyclists' cadence at 110 - 120 rpm. For the 60 and 240 s efforts the cyclists self-selected the resistance and their cadence. Only fourteen of the cyclists performed the 600 s effort (cadence data omitted due to a technical error). Cyclists were highly motivated given the context of the tests which were used for the selection purposes. No external motivation was provided by the testers.

Within 24 h of the PP test, cyclists underwent fasted morning measurements of body composition using dual-energy X-ray absorptiometry (DXA; narrowed fan-beam; Lunar Prodigy; GE Healthcare, Madison, WI) and GE Encore 12.30 software (GE Healthcare, Madison, WI) using standardised techniques developed by Nana et al. (2012). The legs of the DXA scans were segmented to give a measure of lower body lean mass (LBLM). The segment for each leg was lateral to a line drawn between the anterior superior iliac spine and the most lateral aspect of the ischium; it extended distally to femoral epicondyles excluding tissue within the pelvic angle (see Figure 6). This segment included most of the quadriceps and hamstrings as well as a small portion of the gluteals without including reproductive and digestive system organs and other tissues mediolateral to the pelvic borders. Data from both legs were summed to give the final measure of LBLM.

Linear regression analysis was used to compare LBLM (kg) to MMP for each of the efforts in the power profile. The linear slopes of the relationship between MMP and LBLM were plotted separately against duration to illustrate the estimated contribution of lean mass to power output. A one-phase decay model was fitted to this using GraphPad Prism software (GraphPad Software, Inc., La Jolla, CA, USA). Unless stated otherwise, estimated means and 95% confidence intervals [95 % CI] are reported. Statistical analyses were conducted using JMP Pro® v10 (SAS Institute Inc., Cary, NC, USA).

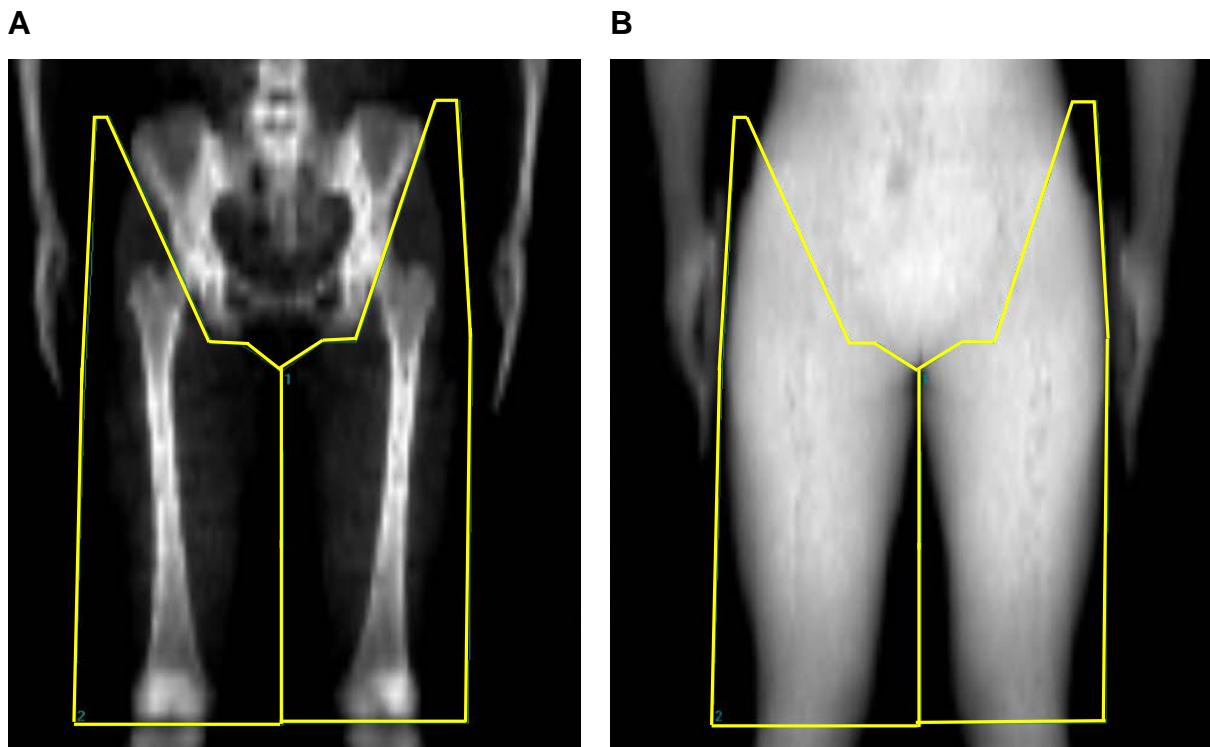


Figure 6. The segmentation of a dual energy x-ray absorptiometry (DXA) scan of the lower limbs for estimation of lower body lean mass (LBLM) with (A) showing the segmentation performed using bone landmarks and (B) showing the segmentation at the gross level including non-lean mass tissues

5.3 Results

Over two selection camps, 33 female cyclists were recruited (24.42 y [22.91, 25.94]; 58.87 kg [58.85, 60.89]; maximal aerobic power 298 W [290, 307]). The mean LBLM was 11.58 kg [11.03, 12.11]. The mean MMP and LBLM data are shown in Table 11. The MMP for efforts of all durations were significantly ($p < 0.05$) correlated with LBLM, however the slope reduced in a curvilinear fashion (Figure 8 A) such that the contribution for efforts ≥ 240 s approaches a plateau at ~ 10 W/kg ($\sim 4\%$) whereas the slope was exponentially greater for shorter durations. To account for scaling issues, the linear regression for each duration was used to estimate the percentage change in MMP for a 1 kg change in LBLM (Figure 8 B).

Table 11. Maximal mean power results from the power profile test expressed as absolute power, relative to body mass and relative to lower body lean mass as a measure of functional lean mass.

<i>Duration</i> (s)	<i>MMP</i> (W)	<i>MMP</i> (W/kg)	<i>MMP</i> (W/kg LBLM)	<i>Cadence</i> (rev/min)
1	835 [791, 879]	14.2 [13.6, 14.8]	72.3 [70.0, 74.5]	115 [111, 118]
5	775 [735, 815]	13.2 [12.6, 13.7]	67.0 [65.0, 69.0]	117 [114, 120]
15	634 [603, 666]	10.8 [10.4, 11.2]	55.0 [57.0, 53.0]	116 [113, 118]
30	510 [484, 536]	8.7 [8.3, 9.0]	44.3 [42.6, 45.9]	112 [110, 115]
60	404 [386, 421]	6.9 [6.6, 7.1]	35.1 [33.9, 36.2]	106 [103, 109]
240	291 [281, 300]	5.0 [4.8, 5.1]	25.3 [24.5, 26.2]	101 [98, 104]
600	264 [254, 274]	4.6 [4.5, 4.7]	22.9 [21.8, 23.9]	-
1800	236 [229, 243]	4.0 [3.9, 4.1]	20.5 [19.9, 21.2]	99 [97, 101]

Results presented as means [95% confidence interval].

Maximal mean power (MMP); lower body lean mass (LBLM); confidence interval (CI).

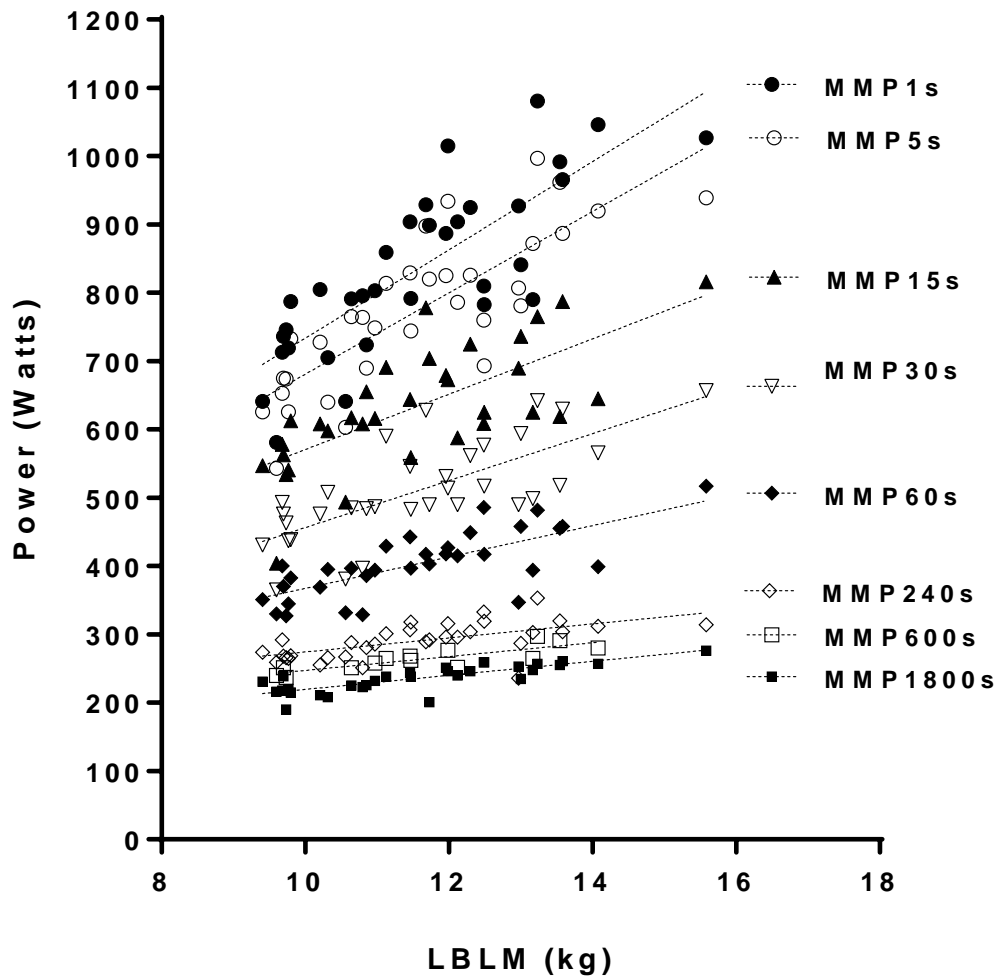


Figure 7. The relationship between Maximal Mean Power (MMP) and lower body lean mass (LBLM) for efforts of increasing duration from the power profile. Closed circles represent peak power output ($Y = 87.3 + 64.62X$; $R^2 = 0.64$); open circles represent the MMP for 5 s ($Y = 85.9 + 59.05X$; $R^2 = 0.65$); closed triangles the MMP for 15 s ($Y = 165.5 + 40.51X$; $R^2 = 0.50$); open triangles the MMP for 30 s ($Y = 112.7 + 34.37X$; $R^2 = 0.53$); closed diamonds the MMP for 60 s ($Y = 138.0 + 22.94X$; $R^2 = 0.53$); open diamonds the MMP for 240 s ($Y = 171.8 + 10.26X$; $R^2 = 0.37$); open squares the MMP for 600 s ($Y = 146.1 + 10.12X$; $R^2 = 0.71$); and closed squares the MMP for 1800 ($Y = 116.8 + 10.28X$; $R^2 = 0.65$).

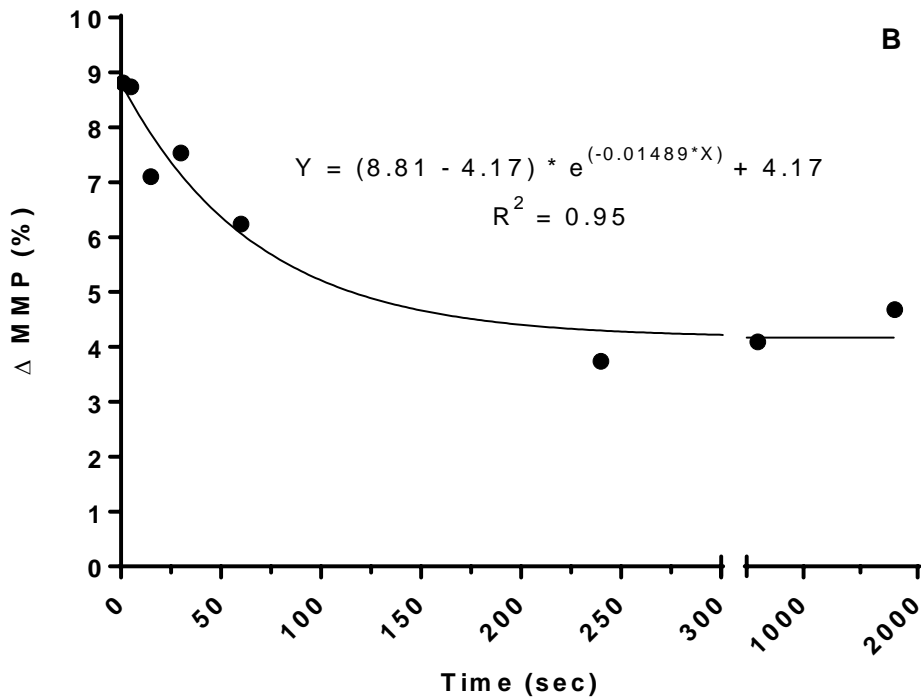
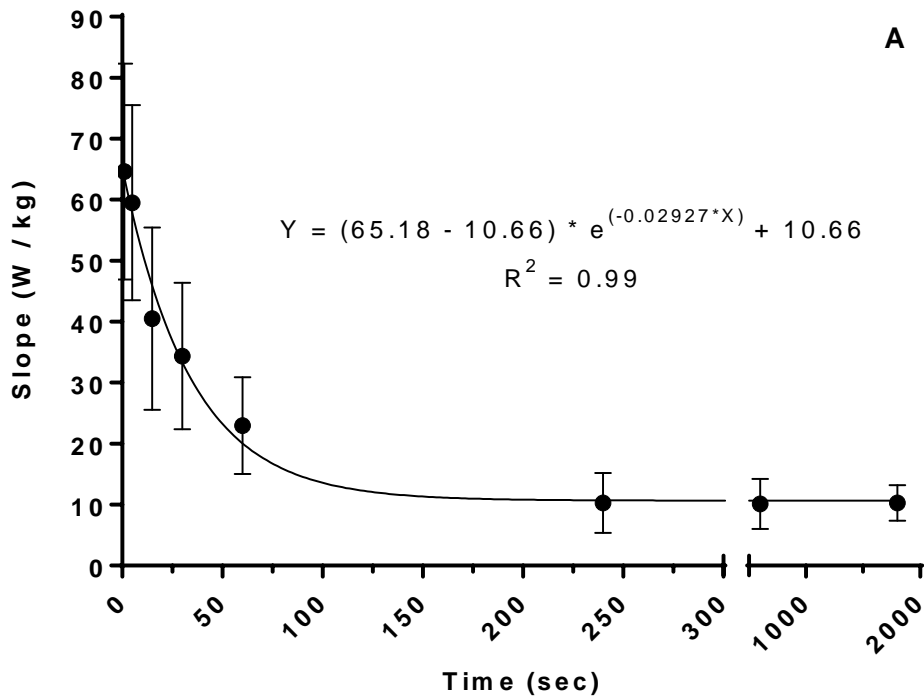


Figure 8 (A) Plots the slope (mean [95% CI]) of the linear relationship between Maximal Mean Power (MMP) and lower body lean mass (LBLM) for maximal efforts of stationary cycling over different durations from the power profile. (B) Plots the estimated percentage change in MMP per kilogram change in LBLM based on the linear regression models.

5.4 Discussion

The purpose of this study was to describe the relationship between MMP and functional lean mass in female road cyclists performing efforts of increasing duration. Across all durations (1 – 1,800 s), the maximal sustainable power output shared a moderate to strong correlation with functional lean mass defined as LBLM. While LBLM explained ~50 - 60% of the variance in maximal mean power (MMP) for most durations, the slope of this relationship declined as duration increased. To account for scaling issues, the estimated relative change (percentage change) in power output attributed to a 1 kg change in LBLM was plotted using the linear regression model for each effort (Figure 8 B). The decline in contribution of LBLM to MMP was still curvilinear when presented in this format. This suggests that LBLM has a greater contribution to power output during short maximal efforts of ~60 s or less which has implications for road cycling performance in sprints, lead outs (team mates of the designated sprinter will ride as fast as possible with the sprinter in their draft to lead them at the front of the race) and attacks (a rapid acceleration by a rider to avoid having other riders remain in their draft). In the context of track endurance events it has implications for the individual and team pursuits and the omnium.

Female cyclists are a weight conscious population (Haakonssen et al., 2014a) and road cycling is considered a weight sensitive sport (Mountjoy et al., 2014; Sundgot-Borgen et al., 2013) where athletes are likely to manipulate BW to improve performance. Energy restriction combined with exercise in obese females results in a weight loss composition consisting of ~15 % lean tissue which is mostly muscle mass (Weinheimer et al., 2010). As cyclists strive for low competition weights, muscle mass may be compromised. Using the regression analyses from this investigation with our reference female (58.87 kg; 11.58 kg LBLM), it is possible to predict the performance outcomes associated with functional lean mass losses. A loss of 0.5 kg of LBLM would be predicted to reduce MMP 1 s by ~33 W (3.9%). Of course, total BW would also be reduced so the estimated reduction in power relative to BW would be attenuated slightly; 0.4 W/kg (3.0%). The same reduction in LBLM is predicted to reduce MMP 240 s by ~6 W (1.8%) or 0.05 W/kg (0.9%). To achieve a 1% improvement in MMP 1 s (W/kg), this cyclist would require a further 2.3 kg reduction of non-functional mass (fat, upper body lean mass). Similarly, a 1% improvement in relative MMP 240 s would require a 1.1 kg loss of non-functional mass. Although reductions in non-functional mass may be desirable, a loss of functional mass may compromise cycling power and have a negative impact on performance. Reducing

adipose tissue in isolation is likely to be challenging with traditional weight loss practices. Weinheimer et al. (2010) suggest that the composition of weight loss in energy restricted middle-aged and older adults is approximately 70-80% adipose and 20-30% lean tissue. The magnitude to which lean tissue losses can be attenuated in athletes who are either energy restricted or engaged in novel training or dietary strategies is of interest.

While the data collected in this study are cross-sectional and require further validation, future prospective training studies may identify specific strategies that improve power output relative to LBLM. Data such as that presented in this study may then be used as a reference to help guide specific training interventions. Because of factors that affect joint power other than lean muscle mass, there is likely to be some inherent variability amongst female cyclists not accounted for by lean mass alone. Nonetheless, for female cyclists well above the normative values (high muscle quality), it is possible that the most feasible way to increase power is to further increase LBLM. Strength training that elicits muscle hypertrophy may present as a useful option. In contrast, an athlete with apparently poor muscle quality (low power relative to LBLM) may require sprint training or power based exercises to improve motor unit recruitment and coordination. Further research is required to investigate the efficacy of such individualised strategies.

The findings of this study provide some evidence that for the weight conscious cyclist, strategies that at least maintain functional lean mass during weight loss may be important for performance. A 12 wk training study with female cyclists involving twice weekly resistance training did not increase total BW more so than the control group (Bishop et al., 1999). Likewise, aerobic capacity (maximal oxygen uptake) and endurance (60 min time trial performance) were not impaired. Unfortunately, these investigators did not measure sprint performance or more competition specific measures of performance.

Information regarding previous involvement with strength and/or power training was not recorded in this study. This may be an important covariate given both strength and power training can increase the rate of force production and peak torque, independent of lean mass changes (Coburn et al., 2006; Cormie et al., 2010). It should be noted that strength and power are influenced by numerous factors other than mass or cross-sectional area and these characteristics may adapt at different rates in response to strength or power training (American College of Sports Medicine, 2009). Muscle architecture (Gülch, 1994),

neural function (Sale, 2003) and fibre type (Fitts and Widrick, 1996) will influence strength and power and these factors are not captured by measuring lean mass alone.

In addition to strength training, nutritional strategies such as the inclusion of high biological value proteins, may also promote a favourable body composition for cyclists. Guidelines by Areta et al. (2013) indicate that 20 - 25 g of protein should be consumed every ~3 h throughout the day to enhance muscle protein synthesis. There is also evidence that when dairy is consumed in an energy restricted state, weight loss is accelerated and a greater proportion of weight lost is adipose (Zemel, 2004).

In this study, efforts were made to control cadence during the 15 s and 30 s efforts. To more accurately predict maximal instantaneous power output during the 6 s effort, inertial load testing methods described by Martin et al. (1997, 2000) should be used. The inertial load protocol allows peak instantaneous power to be measured through a range of cadences. It also identifies the optimal cadence for peak power output which can then be used to perform isokinetic testing for 5 s and 15 s efforts. Optimal cadence can also act as a surrogate for muscle fibre type, which alongside functional lean mass, helps describe the variability in maximal instantaneous power output. Future studies should employ such methodology to avoid results that for short sprint efforts may be confounded by load or cadence.

Not all athletes and sport scientists have access to expensive imaging techniques such as DXA or indeed MRI. It is possible to utilise anthropometric methods to estimate thigh muscle volume (Martin et al., 2000; Tothill and Stewart, 2002). Many cyclists now have power meters fitted to their bicycles which may be used as a surrogate for expensive ergometers. To further improve the practicality of monitoring thigh volume and power output relationships, it may be possible to utilise standardised road cycling performance tests in concert with anthropometric measures.

5.4.1 Conclusion

Successful world class female cyclists tend to be powerful relative to their BW (Ebert et al., 2005; Martin et al., 2001). Road cyclists sometimes attempt to optimise their power-to-weight ratio by decreasing BW. Our results suggest that lower body lean mass explains a moderate to high proportion of maximal mean power for efforts ranging in duration from 1 s

– 1,800 s. The contribution of lean mass was particularly high for short high intensity efforts of 60 s or less. Weight loss strategies that result in functional lean mass losses may need to be offset by substantial reductions in non-functional lean mass. Weight loss strategies for female cyclists should aim to preserve functional lean mass through diet and training modification.

CHAPTER 6: ENERGY EXPENDITURE OF CONSTANT AND VARIABLE INTENSITY CYCLING: POWER METER ESTIMATES

6.1 Introduction

In cycling, it is desirable for athletes to optimize the power they can produce relative to their body weight (BW). Increased BW increases the cyclist's energy demand to overcome rolling resistance and vertical displacement, and when associated with increases in frontal area, it will increase aerodynamic drag (Olds et al., 1995). This has implications for flat road races, time trialing, and climbing performance. As there is an inverse relationship between mass and acceleration, an increase in BW will also impair sprint performance in cycling. Partly due to these power-to-weight relationships, competitive cyclists frequently manipulate body composition to maximize performance. Martin et al. (2001) have shown that internationally competitive Australian National Team female cyclists tend to be leaner and almost 3 kg lighter than less successful cyclists.

Reducing energy intake (EI) to reduce BW causes a reduction in energy availability (EA), where $EA = EI - EE$ where EE represents exercise energy expenditure. For elite female road cyclists who undertake high volumes of training while trying to maintain a high power-to-weight ratio, there is a risk of excessively reduced EA (<30 kcal/kg/FFM/d), which is known to compromise bone status and reproductive health (Loucks et al., 2011). In this population, exercise typically represents the largest component of total daily EE (Westerterp, 2003). Therefore, accurate measures of exercise EE may be important in guiding appropriate levels of EI to support safe body composition manipulation strategies.

To date, the most common field-based method for estimating EE during cycling has been heart rate (HR); using submaximal laboratory-based HR and $\dot{V}O_2$ relationships, HR in the field can be used to estimate $\dot{V}O_2$ and thus EE (Ainslie et al., 2003). A major limitation to this approach is that HR in the field is influenced by factors other than physical activity such as background diet and environmental conditions (Ainslie et al., 2003). Other

Haakonssen, E.C., Martin, D.T., Burke, L.M., Jenkins, D.G. Energy expenditure of constant- and variable-intensity cycling: power meter estimates. *Medicine and Science in Sports Exercise*. 2013 Sep;45(9):1833-1840.

multisensory devices such as the SenseWear-Armband have been used to capture total daily EE. These are reported to accurately measure EE at rest (Malavolti et al., 2007) and during activities of daily living (St-Onge et al., 2007) but significantly underreport EE during moderate- to high intensity (140 – 340 W) stationary cycling (Koehler et al., 2011).

An alternative to these methods is the use of power meters. Commercially available power meters (e.g., Schoberer Rad Messtechnik; SRM) have been used by athletes and sports scientists in the laboratory and the field to quantify the work performed by cyclists since the early 1980's (Allen and Coggan, 2010; Ebert et al., 2005, 2006; Lee et al., 2002; Vogt et al., 2006). When calibrated, these are accurate to within approximately 2% (Gardner et al., 2004). Using indirect calorimetry (CAL) and stoichiometric equations, an individual's actual rate of EE (kJ/min) can be compared with the corresponding rate of mechanical work performed (kJ/min) on a cycle ergometer. This ratio of work expended to work performed can be expressed as a percentage and is called gross efficiency (GE). If cycling power output in the field is known and the work–EE relationship (GE) established in the laboratory is valid and accurate, then EE may be estimated.

Previously, the human body has been estimated to be 25% efficient during cycling, and the mechanical work performed was simply multiplied by 4 to estimate EE (Vogt et al., 2005). Although some studies have used power meters to estimate the EE of professional cyclists (Martin et al., 2002; Vogt et al., 2005) and several studies have measured GE during constant-intensity cycling (Chavarren and Calbet, 1999; Coyle et al., 1992), research is yet to assess the accuracy of estimating EE during variable intensity cycling that mimics cycling in the field. When the magnitude of EE is high, as it is for cyclists performing high volumes of exercise, subtle errors in estimates of efficiency applied to hours of mechanical work performed, can lead to substantial errors in estimated EE and misguide body composition manipulation strategies. If a valid protocol is used to determine GE, then bicycle power meters could potentially allow for accurate estimates of EE during variable-intensity cycling in the field, and this technology may be a practical tool to guide such strategies.

Accordingly, the primary aim of this study was to compare the effects of constant- and variable-intensity cycling on GE and thus estimates of EE. The secondary aim was to validate the use of commercially available power meters to estimate EE by comparing these estimates to measures made using indirect CAL.

6.2 Methods

The investigation was approved by the Human Research Ethics Committee at the Australian Institute of Sport (AIS). All subjects were informed of testing protocols and risks of the study before providing written informed consent.

6.2.1 Testing protocol

Nine Australian National Team female cyclists (mean \pm SD: 24.2 \pm 3.1 y, 57.7 \pm 5.1 kg) volunteered to participate in the study during the first week of a national team training camp. Each completed an incremental exercise test on a custom-designed wind-braked cycle ergometer (AIS, Canberra, Australia) instrumented with an SRM power meter (Science Version, 8 sg). Cycling began at 125 W and power was increased by 25 W every 3 min until volitional fatigue. Maximal aerobic power (MAP) was calculated as the highest mean power maintained for the last 3 min of the trial. Respiratory gases were analysed as previously described (Saunders et al., 2004), using a custom-designed open-circuit indirect CAL system with associated in-house software (AIS, Canberra, Australia). Briefly, expired air was collected into one of two 150 L bags, which alternated between being filled and being analysed every 30 s. Standard algorithms were used to compute minute ventilation (\dot{V}_E), expired carbon dioxide ($\dot{V}CO_2$), oxygen uptake ($\dot{V}O_2$), and respiratory exchange ratio (RER) from the sum of two consecutive 30 s samples. Calibration of this system has been described elsewhere (Saunders et al., 2004). $\dot{V}O_{2peak}$ was defined as the highest volume of oxygen uptake athletes attained during two consecutive 30 s sampling periods. In our laboratory, this technique has a typical error (TE) of <2.4%. At least 48 h after the MAP test, cyclists completed the first of two trials using the same ergometer described previously. As shown in Figure 9, both trials began with the GE test ($GE_{test} = 4$ min at ~45%, ~55%, ~65%, and ~75% MAP) followed by 2 min at ~45% MAP then 10.5 min of either constant- or variable-intensity cycling averaging ~55% MAP. The variable power trial (VAR) involved 1 min at ~45% MAP and 30 s at ~75% MAP repeated seven times (10.5 min). The constant power trial (CON) involved cycling at ~55% MAP for 10.5 min. Immediately after CON or VAR trials, athletes completed a second GE_{test} . For ecological validity during the GE_{test} and for both the CON and VAR trials, the athlete self-adjusted their power output by altering pedalling rate (thereby increasing fly wheel speed), by adjusting the ergometer's infinite gearing system, or by a combination of the two methods. The range of intensities used (45 – 75% MAP) was selected on the basis of

prior analysis of 9 mo of SRM power meter training files from four National team female cyclists. This showed that $51 \pm 3\%$ of all work (kJ) performed during training occurred between 45% and 75% of MAP, and $80 \pm 0.02\%$ is performed below 75% MAP. The shortest interval during the VAR trial was set at 30 s because this corresponded with the 30 s sampling rate of the indirect CAL system. Expired air was collected during all trials and analysed using the same procedures as those used in the MAP test. The two trials were separated by no more than 7 d and were conducted at approximately the same time of the day. Preparation was standardized by ensuring that the same training session was conducted on the day before the trial and that no further exercise was undertaken on the testing day before the testing session. Cyclist and trial order were randomized.

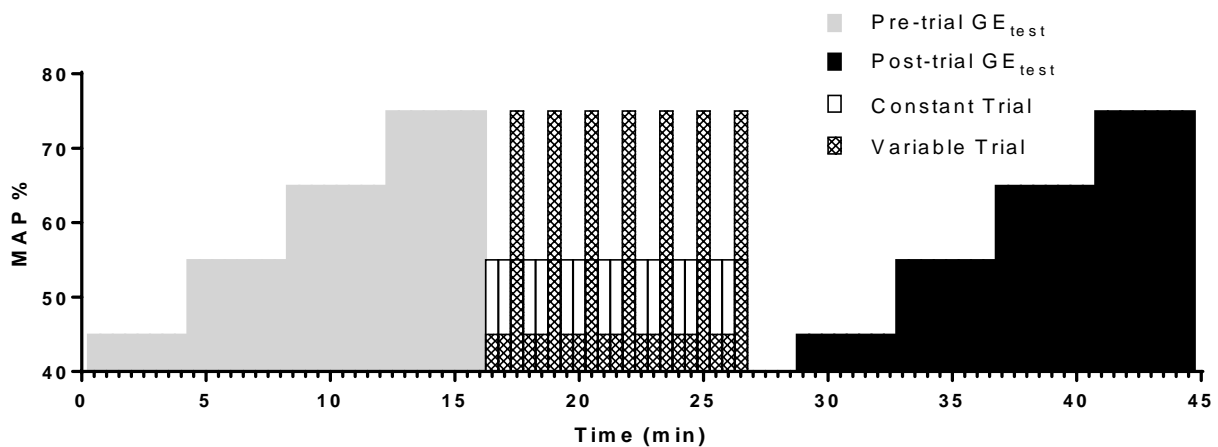


Figure 9. Schematic overview of gross efficiency testing (GE_{test}) protocols. The two testing protocols comparing the effects of constant and variable intensity cycling on gross efficiency begin with a Pre-trial gross GE_{test} (grey columns) followed by 10.5 min of either constant (transparent columns) or variable (crossed columns) intensity cycling, 2 min at 45% maximal aerobic power (MAP) and finally the Post-trial GE_{test} (solid black columns).

6.2.2 Power meter data collection

The SRM power meter was calibrated using a custom-designed dynamic calibration rig (AIS, Canberra, Australia), which has been previously described (Abbiss et al., 2009; Gardner et al., 2004; Maxwell et al., 1998; Woods et al., 1994) with the slope being adjusted until the mean residual power was less than 2% across a range of 80 to 600 W at 100 rpm. During trials, the SRM computer was set at a sampling rate of 2 Hz, and the offset was zeroed after the crank had been used for approximately 15 min and before the

start of all trials. After each trial, data from the SRM computer were downloaded with the actual 30 s mean power being recorded. These data were used for all calculations involving power and gas analysis data including GE.

6.2.3 Data analyses

The relationship between the cyclist's power output and their respiration was measured in two ways: 1) $\dot{V}O_2$ at 150 W ($\dot{V}O_2@150W$) was calculated using each individual's power to $\dot{V}O_2$ regression; and 2) mean GE was calculated as the ratio of rate of work performed (kJ/min) to rate of EE (kJ/min), which was calculated from $\dot{V}O_2$ and RER using the thermal equivalents presented by Lusk (1924). The log-transformed TE (SD of the difference scores divided by square root of two) was calculated for the pre-CON and pre-VAR GE_{tests} to determine the reliability of the testing protocol and the minimum detectable change in GE.

A linear mixed effects model for GE was fitted with time (pre- or post-trial), exercise type (CON or VAR), and MAP% (45%, 55%, 65%, 75%) as fixed effects. Random effects for subject and trial (nested within subject) were fitted. The model was run using a restricted maximum likelihood estimation (REML) method with JMP v7 (SAS Institute Inc., Cary, NC, USA) statistics software. The estimates of the variance components were calculated to describe the unexplained variation (after accounting for the fixed effects) at each of the levels. The subject variance component quantifies the unexplained variation at the subject level, whereas the trial within-subject variance component quantifies the unexplained variation at the trial level. The residual variance component quantifies the unexplained variation among observations within a trial, within a subject. Tukey's HSD post hoc analysis was used where significant interactions were identified.

A series of a priori planned contrasts were made between mean GE, power output (W), cadence (rpm), HR (beats/min), rate of oxygen consumption ($\dot{V}O_2$; L/min), RER, and rate of EE (kJ/min) measured during the CON and VAR trials. These respective comparisons were made using the mean difference and the 90% CI - tested with two-tailed Student's t-tests using spreadsheets created by Hopkins (2006). Cohen's effect sizes (ES) were also calculated. EE (kJ) during the VAR trial was established using the data from indirect CAL as the criterion measure. This was then compared with 10 power meter-based estimates of EE: 1) using the individual's power to GE regression established during the Pre-CON

GE_{test}; 2) using the regression established during the Post-CON GE_{test}; 3) using the regression established during the Pre-CON and Post-CON GE_{tests} combined; 4) using the individual's mean GE calculated during the Pre-CON and Post-CON GE_{tests} combined; 5) using the group's mean GE calculated during the Pre-CON and Post-CON GE_{tests} combined; and 6–10) using the first five methods but applied to the data from the VAR GE_{tests}. To determine how best to use SRM power meter data to quantify EE, the 10 estimates described previously were compared with EE measured using CAL by using the mean difference and the 90% CI. These were assessed using a one-way analysis of variance (ANOVA) and magnitude based inferences (Batterham and Hopkins, 2006). Spearman correlation coefficients were calculated, as well as the range in difference scores expressed as a percentage of CAL. Alpha was set at 0.05.

6.3 Results

The mean (\pm SD) $\dot{V}O_2$ max for the group was 64.2 ± 4.0 ml/kg/min and MAP was 287 ± 23 W. All nine cyclists completed both of the trials and RER did not exceed 1.0 at any point. Some athletes found it technically difficult to maintain the prescribed power output; however, the mean difference (\pm SD) between each cyclist's highest and lowest average power held during a given stage of the GE_{test} was 7.4 ± 2.8 W. GE was highly variable within the group ranging from 16.0 to 21.2% throughout the four GE_{tests}.

6.3.1 Reliability of the test

The regression between $\dot{V}O_2$ and power (W) was strong - $R^2 > 0.98$ in all GE_{tests}. TE (mean \pm 90% CI) calculated from pre-CON and pre-VAR GE_{test} log-transformed data reflects reliable measurements of $\dot{V}O_2$ @150W ($2.4 \pm 2.1\%$) and GE ($2.0 \pm 1.7\%$).

6.3.2 Effect of constant and variable cycling on the gross efficiency test

The subject variance component estimate (\pm SE) from the linear mixed effects model was 1.35 ± 0.70 , which accounted for 81% of the total, whereas the trial within-subject variance component estimate was 0.09 ± 0.06 (5%) and the residual variance component estimate was 0.22 ± 0.03 (13%). Comparison of the GE_{tests} showed that there was no effect of exercise type ($p = 0.74$) on GE and no interactions between exercise type, MAP%, and time ($p = 0.58$). There was a fixed effect of time because GE was reduced from $19.1 \pm$

0.4% to $18.7 \pm 0.4\%$ (mean \pm SE) from pre- to post-trials ($p < 0.0001$). In addition, there was a fixed effect of MAP%, with GE increasing significantly at 65% and 75% MAP compared with 45% and 55% ($p < 0.001$; Figure 10). There was also an interaction found between time and MAP% ($p = 0.0045$). Post hoc analysis revealed that GE was reduced (mean \pm SE: -0.88 ± 0.16) from pre- (18.98%) to post-trial (18.10%) GE_{tests} during the 45% MAP stage only ($p < 0.001$). Additional analysis revealed that cadence had a positive association with MAP% in all GE_{tests} ($R^2 > 0.66$, $p < 0.0001$); however, cadence at any given workload during the GE_{tests} was not significantly different (Table 12).

Table 12. Variability of cadence (rpm) during gross efficiency tests

MAP%	Pre-CON ($\bar{X} \pm SD$)	Post-CON ($\bar{X} \pm SD$)	Pre-VAR ($\bar{X} \pm SD$)	Post-VAR ($\bar{X} \pm SD$)	P
45	90 \pm 3	90 \pm 3	90 \pm 4	89 \pm 3	0.87
55	97 \pm 3	96 \pm 4	97 \pm 2	95 \pm 3	0.38
65	101 \pm 4	100 \pm 4	101 \pm 3	100 \pm 3	0.75
75	104 \pm 5	103 \pm 4	102 \pm 3	102 \pm 4	0.63
(R^2)	0.67	0.66	0.67	0.70	

Results are presented as mean (\bar{X}) and standard deviation (SD). Maximal aerobic power (MAP); constant power output trial (CON); variable power output test (VAR).

6.3.3 Comparison of work and physiological strain during constant and variable cycling trials

As shown in Table 13, none of the variables measured during the CON and VAR trials were statistically different from each other. Gross efficiency during the CON ($18.4 \pm 1.6\%$) and VAR cycling trials ($18.6 \pm 1.1\%$) was very similar ($p = 0.27$); Cohen ES was 0.20. Values for mean power ($p = 0.21$) and cadence ($p = 0.11$) were not significantly different between CON and VAR trials, although the change scores ranged from -5 to 9% and -6 to 3%, respectively. From CON to VAR, there was a worthwhile (ES > 0.2) increase in power (ES = 0.25), HR (0.33), and RER (0.99) and a worthwhile decrease in cadence (-0.44).

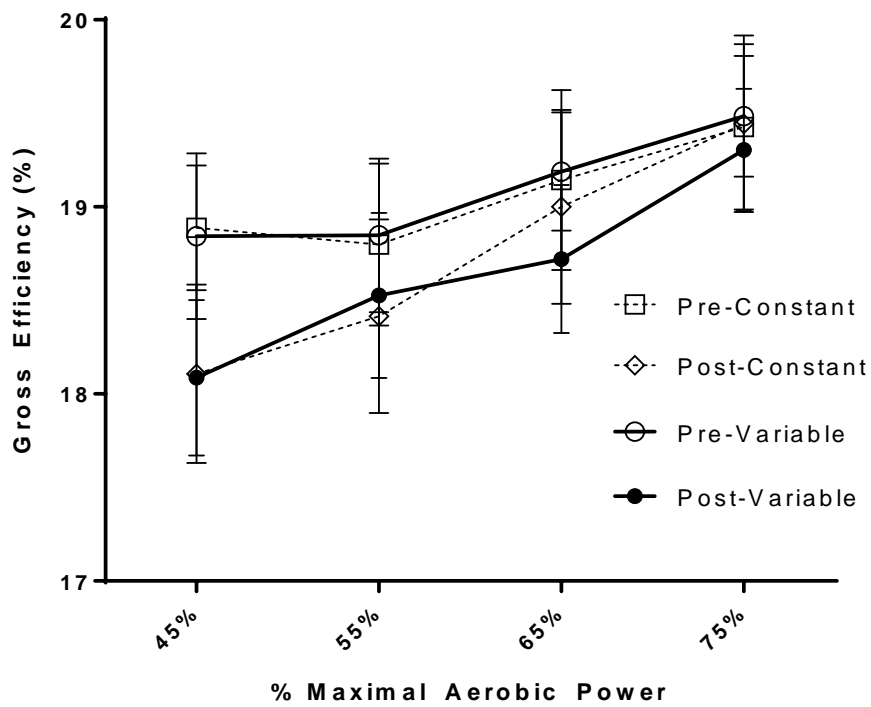


Figure 10. Gross efficiency (GE) measured at 45, 55, 65 and 75% of maximal aerobic power (MAP) before (Pre-) and after (Post-) 10.5 min of constant and variable intensity cycling

Table 13. Measures of work and physiological strain during constant (CON) and variable (VAR) power output trials

	CON ($\bar{X} \pm SD$)	VAR ($\bar{X} \pm SD$)	$\Delta\bar{X} \pm 90\%$ CI (%)	Cohen's Effect Size	P
Power (W)	158 ± 13	162 ± 12	2.3 ± 3.1	0.25*	0.21
Cadence (rpm)	96 ± 4	95 ± 3	-1.7 ± 1.8	-0.44*	0.11
HR (beats/min)	140 ± 11	143 ± 8	2.5 ± 4.7	0.33*	0.34
$\dot{V}O_2$ L/min	2.54 ± 0.24	2.53 ± 0.19	-0.3 ± 4.3	-0.03	0.90
RER	0.85 ± .04	0.89 ± .02	3.9 ± 2.1	0.99*	0.01 [†]
EE (kJ/min)	51.8 ± 4.9	51.9 ± 3.9	0.3 ± 4.1	0.04	0.88
GE (%)	18.4 ± 1.6	18.6 ± 1.1	1.6 ± 2.6	0.20	0.28

Results are presented as mean (\bar{X}) and standard deviation (SD).

Respiratory exchange ratio (RER); oxygen uptake ($\dot{V}O_2$); energy expenditure (EE); heart rate (HR); $\dot{V}O_2$ (oxygen uptake); respiratory exchange ratio (RER); energy expenditure (EE); gross efficiency (GE).

*Cohen's Effect size > 0.2 indicates a worthwhile change.

[†]Significantly different ($p < 0.05$).

6.3.4 Power meter–based estimates of energy expenditure

The total EE measured using CAL for 10.5 min of VAR cycling is shown in Table 14. Comparisons between the criterion and the 10 different estimates for EE during this bout of exercise are also shown in Table 14. Only the estimate using the individual power to GE regression from the Pre-VAR GE_{test} was significantly different to CAL ($p = 0.02$), whereas the regression for the Post-VAR GE_{test} had a similar trend ($p = 0.05$). None of the other estimates were significantly different to CAL, although the range in difference scores (Δrange) using the group mean GE for both the VAR and CON GE_{tests} was approximately 11%, whereas estimates based on the individual regressions for Pre- and Post-VAR GE_{tests} combined and the individual mean for Pre- and Post- VAR GE_{tests} combined reduced the greatest margin of error to <3%. This is further supported by the qualitative inferences. Given that the VAR-based methods generally had a better agreement with CAL, the individual estimates were plotted against CAL measurements in Figure 11 - the diagonal line illustrates a perfect relationship with the criterion measure. Based on the

ANOVA, the Pearson correlation, the magnitude of error, and the qualitative inferences, the combined Pre- and Post-VAR individual regression appears to be the most accurate method, whereas the group mean methods were much less accurate.

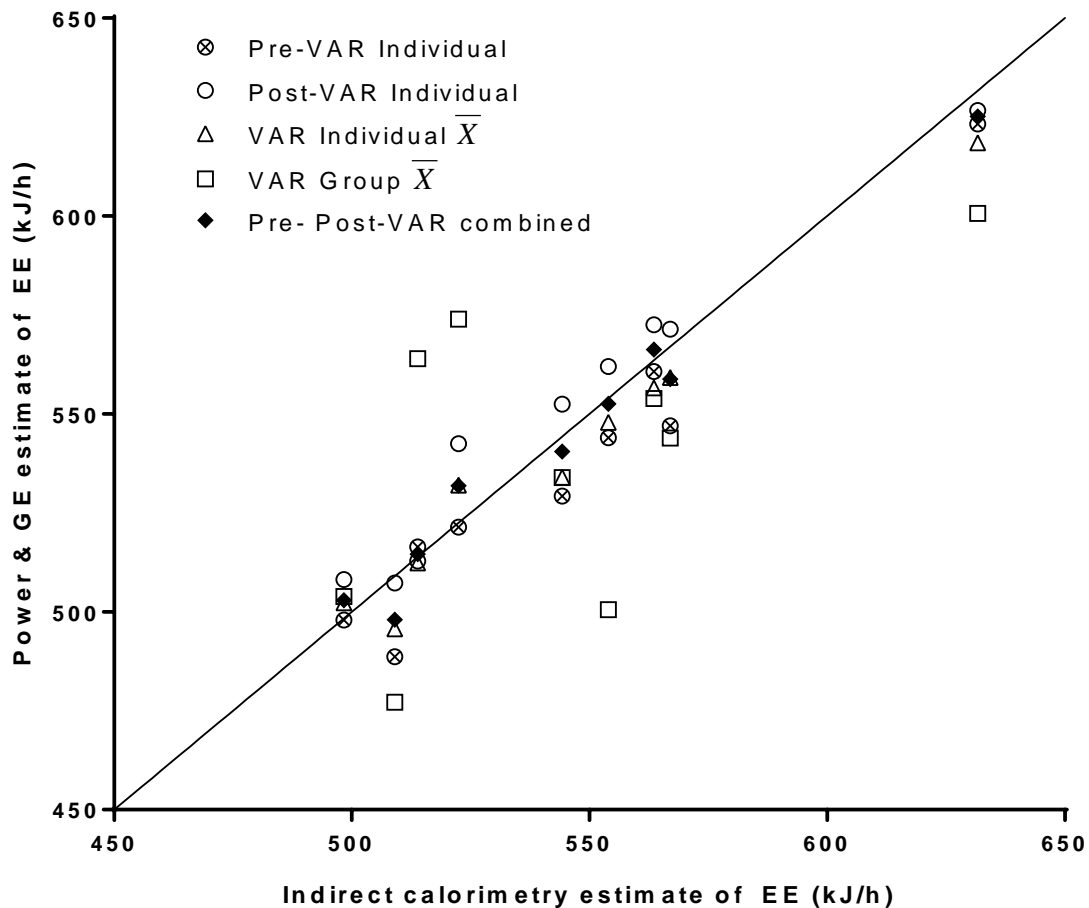


Figure 11. The relationship between energy expenditure (EE) measured using indirect calorimetry during the variable trial and estimates based on the variable intensity (VAR) gross efficiency test (GE_{test}) data. Estimates were calculated using the group mean gross efficiency (GE; open squares); each athlete's mean GE (open triangles); each athlete's GE calculated using their individual power to GE regression from Pre-VAR (crossed circles) and Post-VAR GE_{test} (open circles); and GE from the combined Pre-VAR and Post-VAR regression (solid diamonds). The solid line represents a perfect correlation.

Table 14. A comparison of estimates of energy expenditure during a 10.5 min bout of variable-intensity cycling (average power output of 55% maximal aerobic power) compared with the criterion measure - indirect calorimetry. The estimates are made using both regression equations (for power output and gross efficiency) and mean values from gross efficiency tests performed before and after bouts of constant- (CON) and variable- (VAR) intensity cycling

Method of Estimate	Indirect Calorimetry	Individual Power / GE regression			Individual \bar{X} GE	Group \bar{X} GE	Individual Power / GE regression			Individual \bar{X} GE	Group \bar{X} GE
		Pre-CON GE_{test}	Post-CON GE_{test}	Pre- Post-CON GE_{tests}	Pre- Post-CON GE_{tests}	Pre- Post-CON GE_{tests}	Pre- VAR GE_{test}	Post- VAR GE_{test}	Pre- Post- VAR GE_{tests}	Pre- Post- VAR GE_{tests}	Pre- Post- VAR GE_{tests}
EE (kJ) $\bar{X} \pm SD$	545 ± 41	538 ± 40	551 ± 41	544 ± 40	540 ± 39	539 ± 39	536 ± 40	551 ± 39	543 ± 39	540 ± 37	539 ± 39
$\Delta\bar{X}$ (%) ± 90% CI	-	-1.3 ± 2.0	1.1 ± 2.3	-0.2 ± 2.0	-1.0 ± 2.0	-1.2 ± 4.2	-1.5 ± 1.0	1.1 ± 0.9	-0.3 ± 0.8	-1.0 ± 0.9	-1.1 ± 4.2
Range of residuals (%)	-	-6.6 – 2.5	-3.3 – 6.3	-4.6 – 4.4	-5.8 – 3.8	-10.8 – 8.9	-4.2 – 0.5	-0.8 – 3.7	-2.2 – 1.8	-2.7 – 1.8	-10.7 – 9.0
ANOVA (P)	-	0.24	0.42	0.88	0.37	0.61	0.02 [†]	0.05	0.54	0.10	0.64
(R ² ; P)	-	0.83; 0.001	0.77; 0.002	0.82; 0.001	0.84; 0.001	0.35; 0.091	0.96; <0.001	0.97; <0.001	0.98; <0.001	0.97; <0.001	0.35; 0.091
Qualitative Inference*	-	Likely trivial (0/91/9)	Likely trivial (8/91/1)	Very likely trivial (1/97/2)	Very likely trivial (0/95/5)	Possibly trivial (5/72/22)	Very likely trivial (0/98/2)	Most likely trivial (0/100/0)	Most likely trivial (0/100/0)	Most likely trivial (0/100/0)	Possibly trivial (5/73/21)

Mean (\bar{X}); standard deviation (SD); confidence interval (CI); gross efficiency (GE); energy expenditure (EE)

*(% chance of the difference between the estimates and the criterion measure excessively high / trivial / excessively low).

[†] Significantly different to Indirect Calorimetry (p < 0.05).

6.4 Discussion

The main finding from this study was that variable intensity cycling had no discernible effect on gross efficiency (GE) or estimated energy expenditure (EE) when compared with constant-intensity cycling. Gross efficiency was almost identical during an approximately 10 min bout of constant- (CON; $18.4 \pm 1.6\%$) and variable- (VAR; $18.6 \pm 1.1\%$) intensity cycling. Gross efficiency measured using a reliable (mean \pm 90% CI TE: $2.0 \pm 1.7\%$) GE testing protocol (GE_{test}) was affected by exercise intensity (MAP%) before and after CON and VAR cycling and time (pre- or post-trial) but not by exercise type (CON or VAR). This suggests that elite female cyclists become less efficient after prolonged submaximal (<75% MAP) cycling, but the variability of the intensity has no effect on GE and thus EE. Although VAR cycling did not appear to affect the relationship between power and $\dot{V}O_2$ (L/min) or GE, it is noteworthy that RER was greater during the VAR (0.89 ± 0.02) trial compared with the CON trial ($0.85 \pm .04$, $p = 0.01$). This suggests a greater reliance on carbohydrate metabolism during VAR and possibly a slightly greater anaerobic contribution. This may be caused by the frequent accelerations produced during the VAR trials.

The positive relationship between exercise intensity and GE is expected and well documented (Ettema and Lorås, 2009). This is likely due to the diminishing relative contribution of basal metabolic rate (the non-zero offset) to total EE at higher workloads. It could be argued that delta or net efficiency are more appropriate measures of efficiency given that they account for this offset; however, for the purpose of this study, the interest is in estimating total body EE during cycling, and as such, GE was used because it incorporates EE not directly associated with producing power on a bicycle.

Gross efficiency was consistently lower (mean \pm SE; $-0.4 \pm 0.09\%$ unit change) after the CON and VAR trials ($p < 0.0001$). Most of this change is likely accounted for by the reduction in GE at 45% MAP post-trial ($-0.88 \pm 0.16\%$ unit change), which is indicative of excess oxygen consumption after the 10 min at 55% MAP. These findings are similar (albeit more subtle) to those of Sahlin et al. (2005), who showed that GE at 75% $\dot{V}O_{2\text{peak}}$ is reduced after a series of approximately 2 min supra-maximal efforts (110% $\dot{V}O_{2\text{peak}}$) and Passfield and Doust (2000), who observed a reduction in GE after 1 h of moderate-intensity cycling.

The secondary aim of this study was to validate the use of SRM power meters for estimating EE. Estimates of EE during the VAR trial using indirect CAL were used as the criterion measure against which other estimates were compared. Estimates of EE for VAR cycling using group mean GE from either CON or VAR GE_{tests} yielded results that were acceptable for the group but produced individual errors as great as approximately 11% compared with CAL. Where laboratory testing is not an option, a mean GE of 19% (group mean GE) can be used for well-trained female cyclists with moderate accuracy. For elite cyclists wanting to achieve a more accurate estimate of EE, it is more appropriate to determine their power to GE regression (as described using the VAR protocol). Although the use of Pre-VAR and Post-VAR GE_{test} regressions appeared to over- and underestimate EE respectively, combining the two data sets (Pre- and Post-VAR GE_{tests}) achieved a significant enhancement of the accuracy (Table 14 and Figure 11). The individual mean GE also provided an accurate measure of EE during variable cycling. These strategies reduced the error for estimates of EE to within approximately 2% of CAL, which is similar to the TE reported for SRM power meters (Gardner et al., 2004) and our open-circuit indirect CAL system (Saunders et al., 2004).

Although there were no significant differences in the rate of EE or the GE during the CON and VAR trials, the use of the GE_{tests} from the VAR trials did produce estimates of EE with closer associations to the CAL estimates than those from the CON GE_{tests} . This may be explained in part by biological variability, whereas the CON GE_{tests} occurred on a separate testing day, the VAR GE_{tests} were undertaken directly before and after the bout of VAR cycling used for comparison. Although it was not possible with the current group of athletes, this methodological limitation could be eliminated by using a third testing day to produce the criterion measure.

Although there was little difference between the accuracy of measurements made using individual power GE regressions and individual mean GE, the regression could be considered more appropriate given the positive association shown between MAP% and GE. A meta-analysis of studies of GE in cycling by Ettema and Lorås (2009) shows that the relationship between work and GE is curvilinear rather than linear, as is perhaps indicated in Figure 10. Although they further suggest that the effect of work rate on GE is negligible beyond 150 W, the studies cited in their article mostly involved male cyclists, and the threshold may be lower in females. If this is the case, there is an argument for simply using the individual athlete's mean GE and applying this to all submaximal power

outputs to calculate EE. When the results from the VAR trial are extrapolated to estimate EE for 1 h of variable cycling with an average intensity of 55% MAP, the individual regression and individual mean GE methods have a maximum error of 63 kJ (mean \pm SD: -9 ± 38) and 76 kJ (-29 ± 44), respectively, for a workload, resulting in an average individual EE of 3114 ± 234 kJ on the basis of CAL. Even using the highest margin of error observed, these differences are clinically insignificant.

Traditionally, HR monitors have been used to estimate EE in cyclists. Keytel et al. (2005) have shown a strong agreement ($R^2 = 0.83$) between HR-based estimates of EE and those made using indirect CAL with an average absolute difference (percentage difference not provided) of 1.06 ± 7.83 kJ/min. This represents an average hourly error (approximately 64 kJ) that is comparable with the greatest margin of error observed using the methods described previously. Other methods for estimating exercise EE include multi-sensor devices such as the SenseWear-Armband. These have been shown to underestimate exercise EE during steady-state stationary cycling at 50% $\dot{V}O_{2\text{peak}}$ (Brazeau et al., 2011) as well as at all intensities of a 5 min (40 W) stage incremental cycle ergometer test (Koehler et al., 2011). At this time, their use may be more suited to the estimation of non-exercise EE in cyclists, which still represents a significant component of total daily EE.

As well as determining an individual's GE, it is important to ensure that the testing ergometer and the power meter are calibrated. The oxygen cost for a given workload display has been shown to vary by -10% and 18% on popular ergometers (Guiraud et al., 2010). The SRM Power meter has an average (\pm SD) factory error of $2.3\% \pm 4.9\%$, which can be reduced to $0.8\% \pm 1.7\%$ by performing a dynamic calibration as described by Gardner et al. (2004). Although this is the preferred calibration method in our laboratory, the more practical and commonly used method is a static calibration whereby a series of known torques are applied to the stationary crank and the slope of the torque frequency relationship is determined. This method is acceptable, having an excellent agreement with the dynamic calibration process ($R^2 = 0.99$) and average difference of 1.20% (95% CI, 0.66). By ensuring accurate power readings on both testing ergometers and in field use, power meters will improve estimates of GE and thus EE.

The difference between subjects for GE was wide with a range of 17.1 – 20.7% during VAR cycling and 16.0 – 21.2% throughout the four GE_{tests} . Reasons for individual

differences are unclear from this study, although previous work describes numerous sources of variability. It has been suggested that GE is trainable, improves over an athlete's career (Coyle, 2005), and is affected by training experience (Hopker et al., 2007). Moseley et al. (2004) showed no differences in GE between a large group ($n = 65$) of recreational, elite, and professional male cyclists and no correlation between $\dot{V}O_2\text{max}$ and GE. Coyle et al. (1992) observed that GE was highly variable in well-trained male cyclists (18.3 – 22.6%) and that most of this variability ($R^2 = 0.56$) could be explained by the percentage of type 1 muscle fibres. Ettema and Lorås (2009) suggest that researchers should allow for approximately 5% biological variability in GE. Early work by Bahr et al. (1991) showed that cycling GE was reduced after a 4 d military training course, which included strenuous exercise, as well as sleep and food deprivation. In contrast, Halson et al. (2002) found no changes in GE or economy after 2 wk periods of normal, intensified, and recovery training in male cyclists, suggesting that fatigue related to prolonged training does not affect GE. One possible source of variation between our subjects, which is known to affect GE, is cadence.

In their review, Ettema and Lorås (2009) showed that some researchers have found a positive relationship between cadence and efficiency, whereas others show an inverse relationship across a cadence range of approximately 60 - 120 rpm. The difference in work rates used across the studies is a likely confounder of any true effect. Furthermore, some studies report a parabolic relationship where there is an optimally efficient cadence (Coast et al., 1986; Ettema and Lorås, 2009; Foss and Hallen, 2005). The relationship remains unclear, but it is estimated that cadence only accounts for approximately 10% of the variability in GE, and the inter-study variability is greater than any clear trend (Ettema and Lorås, 2009). Cadence and crank velocity were deliberately not controlled in this study because gearing in the field is freely chosen and the protocol was intended to replicate road cycling. This study found no clinically or statistically significant differences in cadence during any given MAP% of the GE_{tests} (Table 12) or during the CON and VAR trials. During the GE_{tests} , GE was slightly correlated with cadence ($R^2 = 0.14$, $p = 0.03$) at 55% MAP. This relationship was insignificant at all other intensities and most likely because of the positive association between cadence and MAP%, which resulted from some athletes increasing cadence rather than increasing the ergometer's resistance. Furthermore, variability in cadence between subjects was low, with the greatest inter-individual difference seen during 75% MAP; range: 98–109 rpm. It is unlikely that these differences explained such variability in GE. An additional linear mixed effects model

revealed that there were no fixed effects or interactions between the exercise type (CON or VAR) and cadence for GE ($P > 0.05$).

The only known study to measure EE (and intake) in free living female cyclists is that by Martin et al. (2001). A net efficiency of 24% was applied to power data from these athletes, and this was summed with estimates of basal EE (0.074 kJ/min). Findings from the current study show that accurately calibrated power meters are a useful and valid tool for estimating EE during road cycling. It is clear, however, that individually calculated GE values improve accuracy and may be more practical because they incorporate basal metabolic rate into the measure. The information derived from correct use of power meters has the potential to guide the nutritional practices of cyclists to meet recommendations for EA. Loucks et al. (2011) have suggested a minimum EA requirement of 30 kcal/kg/FFM/d to prevent reductions in basal metabolic rate, impairments of reproductive health, and reductions in bone status. There is also potential for EE derived from power meters to be used in non-athletic populations who wish to lose weight. Cycling is a popular exercise modality, and the ability to quantify the associated energy cost has implications for weight-conscious populations and elite athletes alike.

6.4.1 Conclusion

The mean GE of cycling for national team female cyclists was approximately 19%. Gross efficiency was similar for approximately 10 min of constant- and variable-intensity cycling. Findings support the use of calibrated SRM power meters for estimating cycling EE. For trained, competitive female road cyclists, total mechanical work (kJ) multiplied by 5.3 (assuming 19% GE) provides a reasonable estimation of exercise EE during variable-intensity cycling <75% MAP. Combining SRM power meter data with each cyclist's individually assessed GE greatly improves the accuracy of estimates for EE (<3% error in all cases).

CHAPTER 7: INCREASED LEAN MASS WITH REDUCED FAT MASS IN AN ELITE FEMALE CYCLIST RETURNING TO COMPETITION: CASE STUDY

7.1 Introduction

While the importance of being lean is recognised amongst elite cyclists, little attention has focused on how to best optimise body composition. As such, extreme weight loss techniques have been popularised (Burke, 2001). Loss of body weight (BW) as a result of energy restriction often results in reduced body fat (BF) and lean mass (Krieger et al., 2006). For an athlete, reductions in functional lean mass may be undesirable.

7.1.1 Purpose

Body composition of an elite female cyclist (age 21 y, height 170 cm, mass ~59 kg) recovering from post-viral fatigue was monitored. The intervention objective was to reduce fat and increase lean mass simultaneously while improving health and performance.

7.2 Methods

Body composition was measured using dual-energy X-ray absorptiometry (Nana et al., 2012a). Body weight and skinfolds were measured in duplicate using calibrated calipers (Harpenden West Sussex, England) at seven sites (triceps, subscapular, biceps, supraspinale, abdominal, front thigh and medial calf; Norton and Olds, 1996). Performance was monitored using Maximal Mean Power (MMP) - highest average power output (W) sustained for 60 s (MMP 60 s) and 240 s (MMP 240 s). Maximal Mean Power was measured in the field (race or training) monthly using an SRM (Schoberer Rad Messtechnik, Jülich, Germany) power meter.

Haakonssen, E.C, Martin, D.T., Burke, L.M., Jenkins, D.G. Increased lean mass with reduced fat mass in an elite female cyclist returning to competition: case study. *International Journal of Sports Physiology and Performance*. 2013 Nov;8(6):699-701

7.2.1 Background

The athlete was healthy from pre- (December) to early season (March). Maximal oxygen uptake was 59.7 ml/kg/min; MMP 240 s ranged from 287-300 W, MMP 60 s ranged from 402 - 439 W and body composition was within the athlete's "normal" range (Figure 12). By mid-season (June), MMP 240 s was still high at 303 W; however, shorter anaerobic efforts were not tolerated (MMP 60 s = 379 W) and race performance declined. Despite training modifications, health deteriorated and by late season (August), the athlete was diagnosed with post-viral fatigue. Blood tests (full blood count, iron studies, markers of thyroid, kidney and liver function and inflammation) were all normal. From early to late season, BW increased 3.02 kg and skinfolds increased 18 mm (Figure 12). Fat mass increased 3.84 kg and lean mass decreased 1.36 kg (Figure 13).

7.2.2 Physique manipulation intervention

The athlete completed a 7 d food log pre-intervention and at the start of the intervention. Daily energy intake (EI) was calculated using a software package (FoodWorks® v7; Xyris Software, Queensland, Australia). Cycling energy expenditure (EE) was estimated using SRM power meter data (estimated gross efficiency of ¹20%). Dietary counselling assisted in reducing energy availability (EA = EI – exercise EE) to ~30 - 40 kcal/kg/FFM/d in line with recommendations for reducing body fat (Loucks et al., 2011). After 10 wk, dietary modifications increased EA to ~45 kcal/kg/FFM/d with a goal of continuing to reduce BF while promoting functional protein synthesis, including muscle and haemoglobin (measured using optimised CO-rebreathing technique; Schmidt and Prommer, 2005). Specifically, the dietary plan included increased dairy protein, in the form of 100 ml low fat milk and 150 g yoghurt during breakfast and afternoon snacks. The protein content of the midday meal was increased to ~20 g by the addition of 50 g tuna and 50 g low fat ham to menu choices, and moderate serves of lean protein-rich meats were included at dinner. Meals were adjusted in volume (increased) and energy density (reduced) by adding food items such as cooked and raw vegetables, soups and fruits. Rides ended at times that allowed regular meals to become the recovery meal. Total body resistance training was

¹This study was conducted prior to the study presented in chapter 6 where a protocol for estimating gross efficiency was validated. At this stage, pilot work indicated mean values of ~20% would yield reasonable estimates of energy expenditure during cycling.

performed three times weekly. Bike training included single leg ergometer work and low cadence high force efforts; 'strength endurance'. Low intensity 30 min sessions were performed in a morning fasted state to promote fat utilisation. After 8 wk, ride duration progressed to 5 h twice weekly. During rides ≥ 2 h, a carbohydrate (CHO) intake target of >60 g/h was introduced to optimise exogenous fuel support. Gym sessions and rides ≥ 2 h were immediately followed by 20 g of high quality protein using a liquid meal supplement (PowerBar Protein PlusTM powder, PowerBar, Australia).

7.3 Results

Pre-intervention EA (mean \pm SD) was $\sim 41 \pm 6$ kcal/kg/FFM/d (CHO/Fat/Pro; 481, 71, 143 g) while the early intervention EA was $\sim 35 \pm 6$ kcal/kg/FFM/d (CHO/Fat/Pro; 382, 66, 155 g). There was a continual reduction of BW (2.82 kg) due to a 3.47 kg reduction in BF while lean mass increased by 0.88 kg (Figure 12 and Figure 13). Lean mass increased most in the trunk (0.61 kg) followed by the arms (0.23 kg), with little change in the legs (0.03 kg). Haemoglobin mass increased by 58.7 g (8.4%) in 6 wk (October to December). By November, MMP 240 s was 286 W and MMP 60 s was 394 W.

7.4 Discussion

This case demonstrates that fat mass can be reduced and lean mass increased during modest energy restriction, provided the dietary intake of high quality proteins is increased throughout the day and increased to 20 g following training, and that >60 g/h carbohydrate is consumed during training ≥ 2 h. Improvements in health, haematological adaptations and performance were also supported. Importantly, when EB was restored (October), FM continued to decrease while lean mass was preserved, suggesting favourable changes in body composition without being energy restricted.

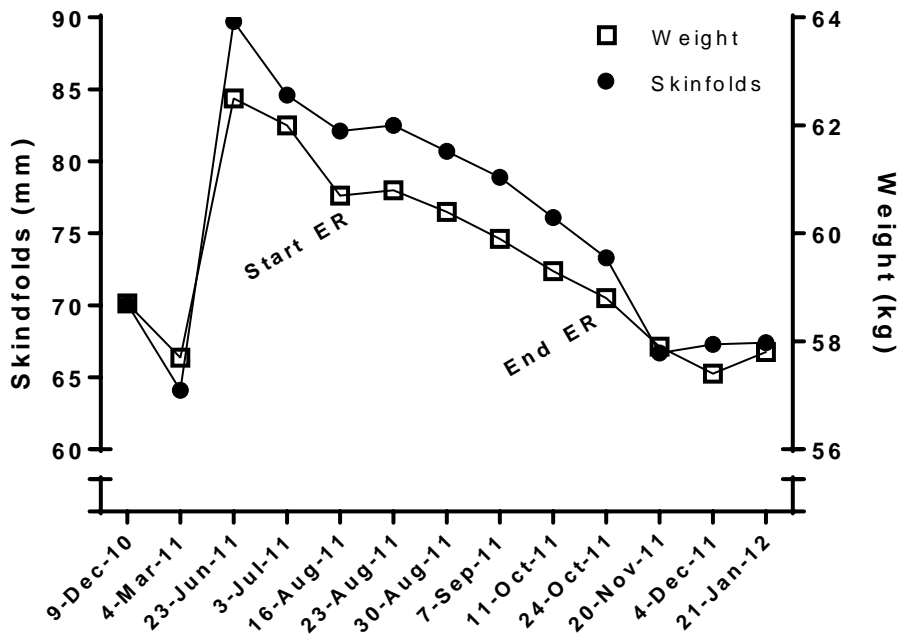


Figure 12. The variation in body weight (open squares) and sum of 7 skinfolds (closed circles) of a female cyclist from pre-season to a midseason period of post-viral fatigue and then during a period of modest energy restriction (ER) from August through October, at which time energy balance was restored.

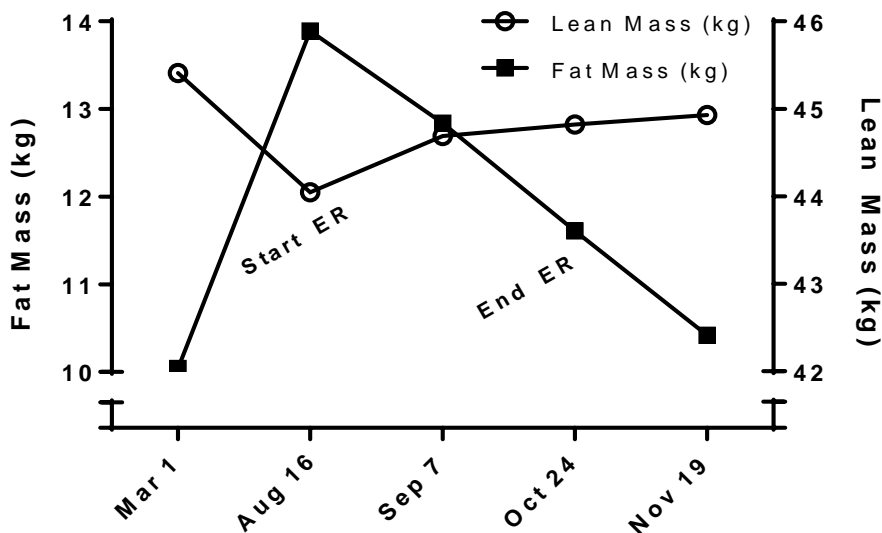


Figure 13. Changes in lean mass (open circles) and fat mass (closed squares) measured using dual-energy X-ray absorptiometry from the early season (March) and then during a period of modest energy restriction (ER) from August through October, at which time energy balance was restored

CHAPTER 8: DAIRY BASED PRE-EXERCISE MEAL DOES NOT AFFECT GUT COMFORT OR TIME-TRIAL PERFORMANCE IN FEMALE CYCLISTS

8.1 Introduction

Endurance athletes may avoid dairy foods in meals consumed prior to high-intensity exercise due to fears about negative side-effects or to preferentially increase carbohydrate intake. Although the perception that dairy foods increase mucous production is disproved (Wüthrich et al., 2005), concerns persist that they may cause gastrointestinal (GI) discomfort (Ray, 2013; de Vrese et al., 2001) thus impairing performance. Exclusion of dairy foods from pre-exercise meals may unnecessarily reduce an athlete's total intake of proteins with high biological value and calcium which may favourably influence body composition (Phillips and Zemel, 2011; Zemel, 2004) and bone health (Cao et al., 2014). Recent investigations have shown that consuming calcium supplements prior to and during exercise may attenuate bone resorption by maintaining calcium homeostasis, which may be perturbed by sweat calcium losses (Barry et al., 2011; Guillemant et al., 2004). Dairy may present an option to increase pre-exercise calcium intake while meeting other sports nutrition goals. Before this is investigated, it is important to ascertain how dairy affects gut comfort and performance.

The aim of this study was to investigate the effects of a dairy-based pre-exercise meal on GI comfort and subsequent exercise performance. A population of well-trained female cyclists was chosen due to our previous observations of their perceptions about dairy foods as well as their known problems with body composition management (Haakonssen et al., 2014a) and bone health (Sherk et al., 2014). It was hypothesised that a dairy based pre-exercise meal would not significantly affect performance or gut comfort following intense cycling. This study was part of a larger investigation of bone turnover and calcium homeostasis in response to exercise (Chapter 9).

Haakonssen, E.C., Ross, M.L., Cato, L.E., Nana, A., Knight, E., Jenkins, D.G., Martin, D.T., Burke, L.M. Dairy based pre-exercise meal does not affect gut comfort or time-trial performance in female cyclists. *International Journal of Sport Nutrition and Exercise Metabolism*. 2014 Oct;24(5):553-8.

8.2 Methods

8.2.1 Subjects

Thirty two healthy, competitive female cyclists [mean \pm SD; 24.3 \pm 4.1 y, 60.9 \pm 7.5 kg, 169.3 \pm 7.0 cm, maximal aerobic power (MAP) 283 \pm 28 W, peak oxygen consumption ($\dot{V}O_{2peak}$) 57.1 \pm 4.9 ml/kg/min] were recruited from the 107 female cyclists registered with Australian National Road Series teams with an average (\pm SD) race attendance of 47 \pm 16. Of these, 33 expressed interest in participating and 25 met inclusion criteria (aged 17 - 32 y; \geq 18 mo racing experience). The remaining participants included an international professional, an ultra-endurance mountain biker and 5 well-trained National club-level cyclists. This study was approved by the Human Research Ethics Committee of the Australian Institute of Sport. Subjects were informed of testing protocols and risks of the study before providing written informed consent.

8.2.2 Baseline fitness test

Maximal aerobic power was determined using an incremental step test on a cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands) starting at 125 W and increasing 25 W every 3 min until volitional fatigue. Maximal aerobic power was calculated as the highest mean power maintained for the last 3 min of the test.

8.2.3 Pre-trial diet standardisation

For 24 h prior to each experimental trial, subjects consumed a standardised pre-packaged diet providing 5.0 g/kg body weight (BW) carbohydrate (CHO), 1.5 g/kg BW protein and 1.5 g/kg BW fat. The first 22 h of the standard diet was supplied in the form of pre-packaged meals, with the pre-trial meal (accounting for the final 2 h) provided to subjects in the laboratory. Individualised menus were prepared accounting for food preferences and intolerances using FoodWorks Professional Edition v6.0 (Xyris Software, Brisbane, Australia), as previously described (Jeacocke and Burke, 2010). In the case of gluten sensitivity ($n = 2$) and lactose-intolerance ($n = 1$), low lactose and gluten-free versions of foods matched for nutrient composition were used. Training was standardised for both pre-trial days. Subjects refrained from alcohol consumption over the 24 h period but followed (and replicated) usual pre-race caffeine habits for the pre-trial meal. Compliance

to the diet, determined from a self-reported checklist, was noted by a dietitian on the morning of the trial.

8.2.4 Pre-exercise meal interventions

This was a randomized, counterbalanced crossover design. The pre-exercise meals were scaled to provide 54 kJ/kg and 2 g/kg BW CHO. The dairy-rich meal (Dairy) consisted of rolled-oats cooked with calcium-fortified (Tricalcium phosphate, Nano-calcium) Anlene milk (Fonterra, Auckland, NZ), yoghurt (Yoplait, Boulogne-Billancourt, France) and additional milk while the Control meal provided oats cooked with water and served with canned fruit and nuts (see Table 15). Since the Dairy meal provided more protein and less fat than Control, the standardised meals were manipulated to match total protein and fat intake over the whole 24 h period (see Table 16). The Dairy meal provided ~1350 mg of calcium and an equivalent of 3 serves of dairy foods. Meals were designed to look similar although cyclists were not blinded to the meal condition given the difficulty of concealing such a high dairy content.

Table 15. Example pre-exercise breakfast for *dairy* and *control* Trials for 60 kg cyclist

<i>Dairy</i>		<i>Control</i>	
Uncle Tobys Quick Oats	57 g	Uncle Tobys Quick Oats	65 g
Anlene Milk	500 ml	Water	250 ml
Yoplait Yoghurt Vanilla	175 g	Goulburn Valley Fruit Salad Diced	140 g
Brown sugar	13 g	Brown Sugar	23 g
Sultanas	20 g	Sultana	23 g
		Macadamias	24 g
		Meadowlea Margarine sachet	15g
		Just Juice, Apple	200 ml

Table 16. Macronutrient composition and calcium content of standardised diets for the *dairy* and *control* trials

<i>Nutrient</i>	<i>24 hour Dietary standardisation</i>		<i>Pre-exercise Breakfast</i>	
	<i>Dairy</i> ($\bar{X} \pm SD$)	<i>Control</i> ($\bar{X} \pm SD$)	<i>Dairy</i> ($\bar{X} \pm SD$)	<i>Control</i> ($\bar{X} \pm SD$)
<i>Energy(kJ/kg)</i>	169 ± 4	170 ± 4	54 ± 2	54 ± 2
<i>CHO (g/kg)</i>	5.1 ± 0.2	5.1 ± 0.2	2.0 ± 0.0	2.0 ± 0.0
<i>Protein (g/kg)</i>	1.5 ± 0.0	1.5 ± 0.0	0.6 ± 0.1	0.2 ± 0.0
<i>Fat (g/kg)</i>	1.5 ± 0.0	1.5 ± 0.0	0.3 ± 0.0	0.4 ± 0.0
<i>Calcium (mg)</i>	1658 ± 174	640 ± 226	1352 ± 53	46 ± 7

Results are presented as means (\bar{X}) and standard deviations (SD). Carbohydrate (CHO).

8.2.5 Exercise protocol

Two hours after starting the pre-trial breakfast, cyclists cycled on an ergometer (Wattbike Ltd. Nottingham, UK) at 60% MAP for 80 min (intensity based on race data from Ebert et al. (2005) followed by a 10 min time trial (TT) during which they were instructed to maintain the highest average power (10 min Maximal Mean Power; MMP 10 min). The TT was limited to 10 min to avoid fatigue for the second trial. The two trials days started 48 h apart to minimise changes in menstrual status. Cyclists were blinded to heart rate, power and cadence but were able to see time remaining. Water was consumed *ad libitum* during the first trial and this hydration strategy was replicated during the second trial. Cyclists consumed a carbohydrate gel (27 g; PowerBar PowerGel, Australia) at 30 and 65 min.

8.2.6 Questionnaires

We developed a Likert questionnaire to check GI comfort during the trial. Cyclists were asked at five time points (before pre-trial meal, 30 min and 60 min after starting pre-trial meal, immediately pre-exercise, and immediately post-exercise) “*how comfortable does your stomach feel at the moment?*”, according to a five point scale (1 = *very comfortable*; 2 = *comfortable*; 3 = *average comfort*; 4 = *uncomfortable*; 5 = *very uncomfortable*). Cyclists

were made aware that gut comfort is a different construct to hunger or satiety and that discomfort on this scale included symptoms such as nausea, bloating and gut pain. After consuming the pre-trial meal, cyclists were asked to indicate how strongly they felt about five criteria relating to that meal (visual appeal, smell, taste, aftertaste, palatability) using a palatability questionnaire (Flint et al., 2000). Responses were recorded on 100 mm visual analogue scales (VAS).

8.2.7 Data analyses

The effect of pre-trial meal type on MMP 10 min was investigated using a linear mixed model with meal (Dairy or Control) as a fixed effect and subject as a random effect. Fisher's exact test was used to assess the association between pre-trial meal gut comfort and meal type. Post-meal gut comfort responses were normalised to the pre-trial response. A negative value implied a relative reduction in gut comfort from pre-trial with the converse true for positive value. Fisher's exact tests were used to assess the association between pre-trial meal type and the delta (Δ) scores for each of the four post-meal time points. To examine the difference in VAS palatability scores between the two meal types, Student's paired t-tests were used for each of the palatability criteria. Statistical analyses were conducted using JMP Pro® v10 (SAS Institute Inc., Cary, NC, USA).

8.3 Results

The mean MMP 10 min was 4 W higher for Dairy compared to Control (95% CI; -2 – 9 W). This difference is neither statistically significant ($t_{31}=1.35$, $p=0.19$) nor clinically meaningful since it is less than the margin of reliability (~2%) of the Wattbike at these power outputs (Hopker et al., 2010). Fisher's exact test showed no evidence of a statistically significant association between pre-trial meal gut comfort and meal type ($p = 0.15$; Table 17), and no evidence of a statistically significant association between gut comfort Δ scores and meal type (Table 17) at 30 min ($p = 0.31$) or 60 min post-meal ($p = 0.17$); immediately pre-exercise ($p = 0.80$) or immediately post-exercise ($p = 0.77$). The estimated differences in mean VAS palatability suggest that for all criteria except for aftertaste, the dairy meal may have been slightly more palatable (Table 18). However, the differences were not statistically significant or clinically meaningful based on a minimal clinically significant difference of 20 mm (Fischer and Singer, 1999).

Table 17. Frequency distribution of gut comfort scores across time points for *dairy* and *control* meal conditions

<i>Pre-Trial</i>	1	2	3	4	5		
<i>Dairy</i>	15	8	6	1	2		
<i>Control</i>	9	16	5	2	0		
<i>Change (Δ) scores</i>							
	-4	-3	-2	-1	0	1	2
<i>30 min Post-Meal</i>							
<i>Dairy</i>	1	2	7	8	10	3	1
<i>Control</i>	0	0	4	7	14	7	0
<i>60min Post-Meal</i>							
<i>Dairy</i>	0	0	5	10	13	3	1
<i>Control</i>	0	1	2	5	14	9	1
<i>Pre-Exercise</i>							
<i>Dairy</i>	0	0	4	13	9	4	2
<i>Control</i>	1	1	4	11	10	5	0
<i>Post-Exercise</i>							
<i>Dairy</i>	1	6	3	7	10	4	1
<i>Control</i>	1	5	4	12	6	4	0

Negative values indicate a relative decrease in gut comfort from Pre-trial values

Table 18. Palatability Scores (VAS) for *dairy* and *control* meal conditions

	<i>Dairy</i> $\bar{X} \pm SD$	<i>Control</i> $\bar{X} \pm SD$	<i>Dairy – Control</i> \bar{X} (95% CI)	<i>T-test</i> statistic; <i>P</i> - value
<i>Visual</i>	27 \pm 27	34 \pm 25	-7 (-17,3)	-1.461; 0.154
<i>Smell</i>	24 \pm 20	30 \pm 20	-6 (-14,1)	-1.740; 0.092
<i>Taste</i>	21 \pm 22	25 \pm 20	-4 (-12,3)	-1.205; 0.238
<i>Aftertaste</i>	53 \pm 31	51 \pm 26	2 (-10,13)	0.285; 0.777
<i>Palatability</i>	23 \pm 20	25 \pm 21	-2 (-10,5)	-0.630; 0.534

Results presented as means (\bar{X}), standard deviations (SD) and 95% confidence intervals (CI).

The Visual Analogue Scale (VAS) was scored so that *Good* was labelled the '0' point of the scale and *Bad* at the '100' point of the scale. A negative score (*Dairy – Control*) indicates a more favourable rating in the *Dairy* trial compared to *Control*.

8.4 Discussion

In contrast to the beliefs of some athletes, we found that consuming substantial amounts of dairy foods in a meal consumed ~90 min before prolonged strenuous stationary cycling, neither impaired nor enhanced gut comfort or performance compared to a control breakfast that excluded dairy foods. Consuming dairy foods during the pre-exercise breakfast should assist in increasing total daily intake of dairy foods and allow the consumption of key nutrients prior to exercise to meet new themes in sports nutrition. Our findings may alleviate concerns expressed by cyclists that consuming dairy in this way would cause gut discomfort leading to performance impairments, at least under the conditions of our study which allowed sufficient time for digestion (~90 min). Possible reasons for GI distress during cycling include ischemic factors that delay gastric emptying and mechanical factors such as increased pressure gradient between the stomach and the oesophagus which may be exacerbated by relaxation of the lower oesophageal sphincter during high intensity exercise (Casey et al., 2005; de Oliveira & Burini, 2009). Our study, the first to investigate the effects of a dairy pre-exercise meal on gut comfort and performance, suggests that this food source does not further increase the incidence of GI distress among cyclists. Further research involving runners and triathletes is warranted, given the higher prevalence of GI distress in these sports (Pfeiffer et al., 2012). It is acknowledged that GI symptoms amongst athletes are variable and some are less likely to tolerate certain foods than others. A limitation of this study is that history of GI distress was not measured and this has been shown to have a strong correlation to GI symptoms reported during exercise trials (Pfeiffer et al., 2009).

The Australian Dietary Guidelines (2013) recommend that females aged 18 - 60 y consume 2.5 serves of dairy per day (one serve = 1 cup milk, 200 g yoghurt or 40 g cheese). These guidelines focus on bone health, where dairy foods are identified as a unique source of well-absorbed calcium. Low bone mineral density is prevalent in both male (Barry & Kohrt, 2008; Campion et al., 2010; Lombardi et al., 2012; Medelliet al., 2009; Nichols et al., 2003; Olmedillas et al., 2011; Rector et al., 2008) and female cyclists (Sherk et al., 2014), possibly owing to the absence of load-bearing exercise (Milgrom et al., 2000) and low energy availability (Barry and Kohrt, 2008; Loucks et al., 2011).

A new hypothesis in sports nutrition involves the specific intake of calcium prior to strenuous exercise to counter sweat calcium loss and its disrupting effects on bone

homeostasis, mediated via alterations in serum ionic calcium levels (Barry & Kohrt, 2008; Barry et al., 2011; Guillemant et al., 2004). There is evidence that supplemental calcium intake before/during cycling attenuates the increase in biomarkers of bone turnover seen in cyclists (Barry et al., 2011; Guillemant et al., 2004). A high calcium dairy-based pre-exercise meal may confer similar bone protective benefits while also meeting other nutritional guidelines.

A further recent theme in sports nutrition is the recommendation that athletes consume an even spread of high quality protein (20 - 25 g of protein per eating occasion) sources every ~3 h over the day to promote optimal muscle protein synthesis (Areta et al., 2013). The inclusion of several serves of dairy foods provides a simple and versatile solution to meeting new protein intake guidelines, particularly at breakfast where they are most likely to be at risk. Indeed, our dairy-rich meal provided ~36 g of protein (60 kg athlete), including a rich source of whey protein which is particularly valuable for muscle protein synthetic needs (Phillips and Zemel, 2011). Although the intake of dairy foods in a pre-exercise meal seems able to target these themes, we considered it important to establish that it was also compatible with other sports nutrition requirements and performance goals.

8.4.1 Novelty statement

Cyclists are known to avoid dairy before exercise in fear of GI discomfort with potential implications for long-term bone health. This is the first study of pre-exercise dairy intake, gut comfort and performance in a representative sample of competitive female cyclists in Australia.

8.4.2 Practical application statement

Cyclists can consume a dairy-rich meal of oats, milk and yoghurt containing ~1350 mg calcium approximately 90 min before starting exercise without any significant effects to gut comfort or performance. This provides key nutrients (calcium and high quality protein) in the total diet as well as specifically prior to exercise.

CHAPTER 9: THE EFFECTS OF A CALCIUM-RICH PRE-EXERCISE MEAL ON BIOMARKERS OF CALCIUM HOMEOSTASIS IN COMPETITIVE FEMALE CYCLISTS

9.1 Introduction

Prevention and treatment of low bone mineral density (BMD) is of high importance for athletes who compete in events where performance is closely related to body composition and situations of low energy availability (EA) frequently arise (Loucks et al., 2011). Cycling is recognised as a sport in which there is a high risk of poor bone health, with BMD found to be lower in cyclists compared to other athletes (Rector et al., 2008) and non-athlete controls (Campion et al., 2010). Average reductions in BMD of ~1.5% have been reported over a cycling season (Barry and Kohrt, 2008) and increases in biomarkers of bone resorption have been detected during a three-week stage race (Lombardi et al., 2012). Findings of low BMD have been observed in male adolescents (Nichols et al., 2003; Olmedillas et al., 2011) and in masters cyclists (Nichols et al., 2003) as well as in professional females within our own lab (Table 21). Risk factors that may underlie these phenomena include a lack of weight-bearing activity (Milgrom et al., 2000) as well as the low EA (Loucks et al., 2011) that occurs due to weight loss practices and/or the high energy expenditure (EE) associated with large volumes of training and racing (Barry and Kohrt, 2008).

Recently, there has been interest in the contribution of dermal calcium losses (sweat calcium loss) during prolonged training sessions to poor bone health. Even if the athlete's diet meets adequate intake (AI) recommendations for calcium and results in calcium balance over the day, the acute and significant dermal calcium losses during exercise may cause a decline in serum ionised calcium concentrations during exercise. Calcium has a vital role as an ion which moves in and out of the cytoplasm, acting as a signal for many cellular processes including exocytosis, neurotransmitter release, muscle contraction and

Haakonssen, E.C., Ross, M.L., Knight, E.J., Cato, L.E., Nana A., Wluka, A.E., Cicuttini, F.M., Wang, B.H., Jenkins, D.G., Martin, D.T., Burke, L.M. Effects of a calcium-rich pre-exercise meal on biomarkers of calcium homeostasis in female cyclists. *PLoS ONE*. [In Press].

the proliferation of action potentials through cardiac muscle. Due to these important functions, serum calcium concentration is defended vigorously within the body. Bone is the largest reservoir of calcium in the body and reductions in serum ionized calcium are therefore mitigated by demineralization of bone - a process stimulated by increases in parathyroid hormone (PTH). Cross linked C-telopeptide of type I collagen (CTX-I) and more recently cross linked C-telopeptide of type II collagen (CTX-II) have been indicated as sensitive markers of osteoclastic bone resorption, while procollagen I N-terminal propeptide (PINP) is indicated as a marker of osteoblastic bone formation (van Spil et al., 2013). Monitoring these markers in addition to PTH can give an indication of bone metabolism in response to exercise and dietary interventions that may disrupt eucalcemia.

Daily calcium supplementation does not appear to provide a clear benefit to BMD per se (Barry and Kohrt, 2008). However, there is some evidence that consuming a calcium supplement in close proximity to or during exercise may reduce the degree to which dermal calcium losses can impair bone health (Barry et al., 2011; Guillemant et al., 2004; Klesges et al., 1996). For example, the consumption of a ~1000 mg calcium supplement prior to (Barry et al., 2011) or during exercise (Barry et al., 2011; Guillemant et al., 2004) has been shown to attenuate exercise-induced increases in markers of bone resorption (Barry et al., 2011). The supplementation protocols in these studies required the consumption of relatively high volumes of calcium fortified water; 1 L consumed 20 - 60 min prior to exercise or 250 ml consumed every 15 min during exercise. In practice, consuming such high volumes of fluid may not be possible or well tolerated by many athletes. The consumption of calcium-rich or fortified foods prior to exercise may confer similar benefits to bone health while also allowing athletes to meet other sports and general nutrition goals. The recommended dietary intake (RDI) of dairy in Australia for females aged 18 - 60 y is 2.5 serves per day (where one serve = 1 cup milk, 200 g yoghurt or 40 g cheese; Australian Dietary Guidelines, 2013).

9.1.1 Project Aims

This study investigated whether the pre-exercise consumption of calcium-rich foods would attenuate exercise-induced perturbations in calcium homeostasis in elite female road cyclists – a population at risk of poor bone health. It was also of interest to investigate any relationships between the magnitudes of exercise induced dermal calcium loss and changes in biomarkers of bone turnover with BMD.

9.2 Methods

9.2.1 Subjects

Thirty-two competitive female cyclists [mean \pm SD; age 24.3 ± 4.1 y, body weight (BW) 60.9 ± 7.5 kg, height 169 ± 7 cm, maximal aerobic power (MAP) 283 ± 28 W, peak oxygen consumption ($\dot{V}O_{2\text{peak}}$) 57.1 ± 4.9 ml/kg/min] participated in the study which was held during a 10 d training camp. The Australian National Road Series had 107 female cyclists registered at the time of this study and an average (\pm SD) race attendance of 47 ± 16 . Of this population, 33 cyclists expressed interest in participating and 25 met the inclusion criteria (17 - 32 y; ≥ 18 mo racing experience; no medical condition affecting calcium homeostasis, able to commit to 10 d camp). The additional participants included an international professional, an ultra-endurance mountain biker and 5 well-trained National club-level cyclists. Exclusion criteria were vitamin D deficiency (25-OH Vitamin D < 30 ng/mL), thyroid dysfunction ($n = 1$), liver or kidney dysfunction, regular use of medications or supplements known to affect bone or calcium metabolism or thyroid function (e.g., thiazide diuretics, bisphosphonates, oral steroids). The investigation was approved by the Human Research Ethics Committee at the Australian Institute of Sport and all subjects were informed of testing protocols and risks of the study before providing signed informed consent. Subjects completed a medical questionnaire and reported having no current injury or illness.

9.2.2 Experimental design

Cyclists performed three exercise trials in a counterbalanced cross-over order separated by one day. Maximal aerobic power was measured at the first visit. In a randomised order, the second and third exercise trials (60% MAP for 80 min followed by a 10 min time trial) were performed ~ 90 min after either a low calcium control meal or a high calcium (~ 1350 mg) dairy based breakfast meal. Blood was sampled on several occasions for further analysis of markers of bone turnover. An overview of the trial days is shown in Figure 14.

9.2.3 Baseline testing

At least 48 h prior to the first experimental trial, MAP, body composition and blood markers were assessed for exclusion criteria. Maximal aerobic power was determined using an incremental step test on an iso-power cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands) starting at 125 W and increasing 25 W every 3 min until volitional fatigue. Maximal aerobic power was calculated as the highest mean power output for the last 3 min of the test. Respiratory gases were analysed, as previously described (Saunders et al., 2004), using a custom designed open-circuit indirect calorimetry system with associated in-house software (Australian Institute of Sport, Canberra, Australia). Bone mineral density (g/cm^2) of the left neck of femur and lumbar spine (L_1 - L_4) was measured by dual-energy X-ray absorptiometry (DXA; narrowed fan-beam; Lunar Prodigy; GE Healthcare, Madison, WI) with analysis performed using GE Encore 12.30 software (GE Healthcare, Madison, WI) using standardised techniques developed by Nana et al. (Nana et al., 2012a).

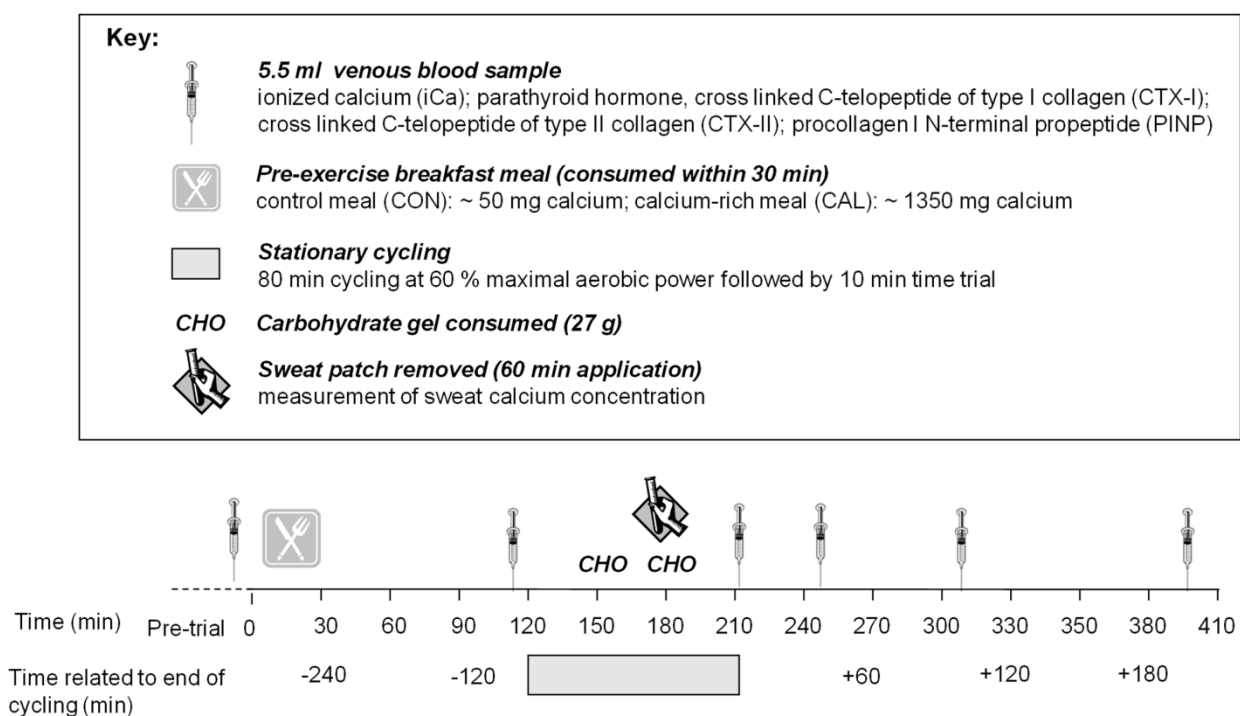


Figure 14. Schematic overview of study design: *The effects of a calcium-rich pre-exercise meal on biomarkers of calcium homeostasis in competitive female cyclists*

9.2.4 Experimental trials

Cyclists presented to the laboratory in a morning-fasted (~9 h) state. Following baseline blood samples (T = -15 min) athletes started the trial (T = 0 min) by consuming a pre-trial breakfast which was either a calcium-rich dairy-based meal (CAL) or low calcium control meal (CON; see *Dietary Standardisation*). Blood was sampled again immediately pre-exercise (T = 115 min); immediately post-exercise (90 min of cycle ergometry; T = 210 min); and at 40 min (T = 250 min), 100 min (T = 310 min) and 190 min (T = 400 min) post-exercise. Sweat samples were also collected after 60 min of exercise to measure sweat calcium concentration.

Subjects were randomised into one of two groups that were balanced for menstrual phase (luteal or follicular which was confirmed with measures of oestrogen and progesterone on trial day one; ovulation was avoided), menstrual regularity (amenorrhea, oligomenorrhea or regular) and use of contraceptives (oral contraceptive pills, contraceptive devices, implants or injections). Trial days began 48 h apart so that changes in the menstrual phase were minimised but allowed for sufficient recovery from exercise and to standardise the 24 h pre-exercise diets. Both trial days started at the same time of day to avoid diurnal variation of biomarkers within subjects.

9.2.5 Dietary standardisation

Subjects followed a standardised diet for 24 h prior to each experimental trial. Individualised menus were based on BW and prepared accounting for food preferences and intolerances using FoodWorks Professional Edition (v6.0, Xyris Software, Brisbane, Australia), as previously described by Jeacocke and Burke (2010). Subjects were provided with all meals and snacks in pre-packaged form for the first 22 h of the standardised period, with the pre-trial meal prepared for subjects upon arrival at the lab 2 h prior to commencing exercise. The standardised diet was designed to provide 5.0 g/kg BW of carbohydrate (CHO); 1.5 g/kg BW of protein; 1.5g/kg BW of fat over the 24 h period. This was inclusive of 2.0 g/kg BW of CHO allocated to the pre-trial meal (see Table 19). In the case of gluten sensitivity (n = 2) and lactose-intolerance (n = 1), low lactose and gluten-free versions of foods matched for nutrient composition were used. Subjects refrained from alcohol consumption over the 24 h period but followed (and replicated) usual pre-race caffeine habits for the pre-trial meal. Compliance to the diet, determined

from a self-reported checklist, was noted by a dietitian on the morning of the trial. Subjects followed standardised training on both pre-trial days (low intensity 1.5 h cycling training).

9.2.6 Pre-exercise meal interventions

The pre-exercise meals were scaled to provide 54 kJ/kg and 2 g/kg BW CHO. The CAL meal consisted of rolled-oats cooked with calcium-fortified (Tricalcium phosphate, Nano-calcium) Anlene milk (Fonterra, Auckland, NZ), yoghurt (Yoplait, Boulogne-Billancourt, France) and additional milk, while the CON meal provided oats cooked with water and served with tinned fruit and nuts (see Table 20). The two trials differed only by the pre-trial meal, with the calcium content of the meals being 1352 ± 53 mg and 46 ± 7 mg for CAL and CON respectively. While attempts were made to ensure the two meals had a similar appearance, it was clear that one contained significantly more dairy and as such subjects were not blinded to the meal condition.

Table 19. Macronutrient composition and calcium content of standardised diets for the *control* and *calcium-rich* trials

<i>Nutrient</i>	<i>24 h dietary standardisation</i>		<i>Pre-exercise breakfast</i>	
	<i>Control</i> ($\bar{X} \pm SD$)	<i>Calcium</i> ($\bar{X} \pm SD$)	<i>Control</i> ($\bar{X} \pm SD$)	<i>Calcium</i> ($\bar{X} \pm SD$)
<i>Energy (kJ/kg)</i>	170 ± 4	169 ± 4	54 ± 2	54 ± 2
<i>CHO (g/kg)</i>	5.1 ± 0.2	5.1 ± 0.2	2.0 ± 0.0	2.0 ± 0.0
<i>Protein (g/kg)</i>	1.5 ± 0.0	1.5 ± 0.0	0.2 ± 0.0	0.6 ± 0.1
<i>Fat (g/kg)</i>	1.5 ± 0.0	1.5 ± 0.0	0.4 ± 0.0	0.3 ± 0.0
<i>Calcium (mg)</i>	640 ± 226	1658 ± 174	46 ± 7	1352 ± 53

Results presented as means (\bar{X}) and standard deviations (SD). Carbohydrate (CHO).

Table 20. Example pre-exercise breakfast meal for *control* and *calcium-rich* trials for a 60 kg cyclist

<i>Control</i>		<i>Calcium</i>	
Uncle Tobys Quick Oats	65 g	Uncle Tobys Quick Oats	57 g
Water	250 ml	Anlene Milk	500 ml
Goulburn Valley Fruit Salad Diced	140 g	Yoplait Yoghurt	175 g
Brown Sugar	23 g	Brown sugar	13 g
Sultana	23 g	Sultanas	20 g
Macadamias	24 g		
Meadowlea Margarine	15g		
Just Juice, Apple	200 ml		

9.2.7 Exercise protocol

Two hours after the start of the breakfast meal, cyclists began exercising on a Wattbike cycle ergometer (Nottingham, UK) at 60% of baseline MAP for 80 min (pre-load) followed by a 10 min time trial. Cyclists were instructed to maintain the highest average power possible for 10 min. The pre-load intensity of 60% MAP was chosen to be challenging enough to significantly increase sweat rate and thereby dermal losses of calcium. The time trial was used to determine whether there was any impact of pre-trial meal on cycling performance, which was previously reported by Haakonssen et al. (2014b). To increase ecological validity, fans were positioned in the same location facing the athlete at a speed of 167 m/s for each trial. Room conditions were controlled at 21 - 22° Celsius and 50 - 60% relative humidity. Water was consumed *ad libitum* during the first trial and this hydration strategy was replicated during the second trial. After 30 min and 65 min of exercise, cyclists consumed a carbohydrate gel (PowerGel, PowerBar, Australia), each providing 27 g CHO.

9.2.8 Blood analyses

On trial days, venous blood was collected at six time-points *via* a cannula: pre-trial (T = -15 min), immediately pre-exercise (T = 115 min), immediately post-exercise (T = 210 min), 40

min post-exercise (T = 250 min), 100 min post-exercise (T = 310 min) and 190 min post-exercise (T = 400 min). Blood was collected into a 2.0 mL BD Vacutainer Plus anaerobic plastic tube (Becton, Dickinson and Company, NJ, USA) to later analyse for ionized calcium (iCa), and into a 3.5 ml BD Vacutainer Plastic SST II Advance tube to measure CTX-I, CTX-II, PINP, and PTH. In trial one, at T = -15, additional blood was collected in a 3.5 ml BD Vacutainer Plastic SST II Advance tube and centrifuged for 10 min at 3000 G for measurement of progesterone and oestrogen.

Blood from the BD Vacutainer Plastic SST II Advance tube was allowed to clot by standing for 2 h at room temperature before being centrifuged at 1000 G for 10 min. Serum (0.3 – 0.5 ml) was then aliquoted into four 0.75 ml polypropylene tubes (Micronic America LLC, Aston, PA, USA). Blood clotting, centrifuge and freeze time were kept consistent for all samples. Once aliquoted, the samples were immediately stored at -80° C. These samples were subsequently used to measure PTH using an Enzyme-Linked immunosorbent Assay (ELISA; IBL International GmbH, Hamburg, Germany); CTX-I using a Serum Crosslaps ELISA (Immunodiagnosics Systems Ltd. Boldon, UK); CTX-II using an ELISA (Novatein Biosciences, Woburn, USA) and PINP using an ELISA (Novatein Biosciences, Woburn, USA). Samples were batched and measured in duplicate using a SPECTROstar Nano microplate reader (BMG Labtech, Ortenberg, Germany) according to the manufacturers' protocols. The intra-class correlation coefficients for the ELISA kits were CTX-I: $r = 0.946$; CTX-II: $r = 0.991$; PTH: $r = 0.996$; and PINP: $r = 0.983$. Sensitivities for the assays were PTH: 1.57 pg/ml; CTX-I: 0.020 ng/ml; CTX-II: 30.0 pg/mL; and PINP: 3.0 ng/ml.

9.2.8.1 Adjustment for haemoconcentration

Changes in biomarkers of bone turnover were adjusted for haemoconcentration using methods described by van Beaumont et al. (1973). This method was used rather than that described by Dill and Costill (1974) because haemoglobin was not measured in this study. Haematocrit (Hct) was measured using the iSTAT analyser (CG8+ cartridge, Abbott Point of Care Inc, Princeton, NJ, USA; CV 1.5% and sensitivity of 10%) and multiplied by a factor (0.96 x 0.91) to correct for plasma trapped between red blood cells and to convert venous Hct to whole-body Hct respectively. Biomarkers were then adjusted to the concentration expected (CE) based on fluid shifts alone. This was done by using the

changes in Hct from immediately pre-exercise (Hct1) to all post-exercise values (Hct2) using Equation 8, where C1 represents the initial concentration of the biomarker.

Equation 8

$$CE = [Hct2(100 - Hct1)/Hct1(100 - Hct2)] \times C1$$

The unadjusted post-exercise biomarker concentrations (C2) were then corrected for CE using Equation 9, giving a Hct-corrected concentration (C2_{Hct}).

Equation 9

$$C2_{Hct} = C2 - (CE - C1)$$

All reported values for PTH, CTX-I, CTX-II and PINP were adjusted for haemoconcentration. Given that the parathyroid responds to the concentration of iCa, results for both unadjusted and adjusted iCa were reported.

9.2.9 Sweat calcium analyses

The regional absorbent patch method was used to collect sweat samples during the experimental trials (Dziedzic et al., 2014). One patch was applied medial to the inferior angle of the right scapula for the purpose of measuring differences in sweat calcium concentration between trials. Baker and colleagues reported that when using regional skin surface sweat collection for the purpose of measuring sweat calcium concentration, no individual site nor the combination of five sites was significantly correlated with a strictly controlled whole body wash down method (Baker et al., 2011). Therefore, only one patch was used in this study. The chosen site was cleaned with distilled water and dried with sterile gauze. An absorbent patch (Tegaderm+Pad, 3M Health Care, Minnesota, USA) was then applied 20 min prior to the start of exercise and removed 60 min into the exercise trial using aseptic techniques and placed into clean filtered centrifuge tubes (Salivette, Sarstedt AG & Co, Germany). After removal, sweat patches were immediately centrifuged at 10° C for 5 min at 3850 G to obtain a sweat sample. Sweat (0.5 mL) was added to 1 mL pooled plasma of predetermined calcium concentration in equal volumes. Sweat ionised

calcium was measured on a blood gas analyser (CV <1%; Siemens RapidLab 1265, AG., Erlangen, Germany).

9.2.10 Data analyses

The study was sized to detect a 20 pg/mL change in the pre-post exercise serum PTH due to the administration of calcium, assuming a SD of the difference of 30 pg/mL, with 95% power at the 0.05 level, using a two-sided test. The effects of different pre-trial meals on Hct, iCa, PTH, CTX-I, CTX-II and PINP were investigated using linear mixed-effects modelling, fit by restricted maximum likelihood estimation (REML) with pre-trial meal, time and their interaction as fixed effects, with subject nested within trial as random effects. All response variables were log-transformed except iCa and Hct which had constant variance. Tukey's HSD post-hoc analysis was used to identify differences. Unless stated otherwise, estimated means and 95% confidence intervals [95% CI] are reported. Differences between means and 95% CI's for log-transformed data were back transformed to obtain estimates of the ratio of medians.

Student's paired t-tests were used to compare total fluid loss and sweat calcium concentration between the two meal conditions. Using data from the control trial, a Spearman's correlation matrix was created to investigate the relationship between dermal calcium loss, total sweat loss and BMD. The relationship between BMD and biomarkers of turnover (post-exercise and pre- to post-exercise change in PTH, CTX-I, CTX-II, PINP and iCa) were investigated similarly. Spearman's correlations between biomarkers of bone turnover (PTH, CTX-I, CTX-II, PINP and iCa) were also calculated. Descriptive statistics and Z and T-Scores were calculated for BMD (g/cm²). Statistical analyses were conducted using JMP Pro® v10 (SAS Institute Inc., Cary, NC, USA).

9.3 Results

Results for Hct, iCa, PTH, CTX-I, CTX-II, and PINP are shown in Figure 15 and Appendix B. There was no interaction between *pre-trial meal* and *time* for Hct ($F_{5, 310} = 1.105$; $p = 0.36$), so this interaction was dropped from the model. There were main effects for *time* ($F_{5, 310} = 75.41$; $p < 0.01$) with Hct increasing on average 2.6 percentage units [1.8, 3.4] from T = 115 min (pre-exercise) to T = 210 min (post-exercise). There were also main

effects for *pre-trial meal* ($F_{5, 310} = 75.41$; $p < 0.01$) with Hct being on average higher in CON (41.4% [40.6, 42.2]) than CAL (40.8% [40.1, 41.6]).

There was a significant interaction between *pre-trial meal* and *time* for unadjusted iCa ($F_{5, 310} = 4.02$; $p < 0.01$). Unadjusted iCa was 0.041 mmol/L higher in CAL than CON at T = 115 min [0.003, 0.078]. Unadjusted iCa significantly decreased by 0.039 mmol/L from T = 115 min to T = 210 min in CON [-0.076, -0.002]. In CAL there was a marginally significant decrease of 0.036 mmol/L [-0.072, 0.001]. At T = 210 min, unadjusted iCa was 0.044 mmol/L higher in CAL than CON [0.006, 0.082] and 0.041 mmol/L higher in CAL than CON at T = 250 min [0.004, 0.079].

There was a significant interaction between *pre-trial meal* and *time* for log(PTH) ($F_{5, 341} = 6.02$; $p < 0.01$) indicating that the effect of meal type changed depending on the time-point. Pre-Ex, the median PTH was 1.33 [1.03, 1.73] times higher in CON than the median PTH in CAL (Figure 15c). Parathyroid hormone increased significantly from T = 115 min to T = 210 min in both pre-trial meal conditions. In CAL, the median PTH at T = 210 min was 1.42 [1.10, 1.84] times higher than the median PTH at T = 115 min, while for CON the median PTH at T = 210 min was 1.66 [1.28, 2.15] times higher than the median PTH at T = 115 min. At T = 210 min, the median PTH was 1.55 [1.20, 2.01] times higher in CAL than CON. At T = 250 min, the median PTH was 1.45 [1.12, 1.88] times higher in CAL than in CON.

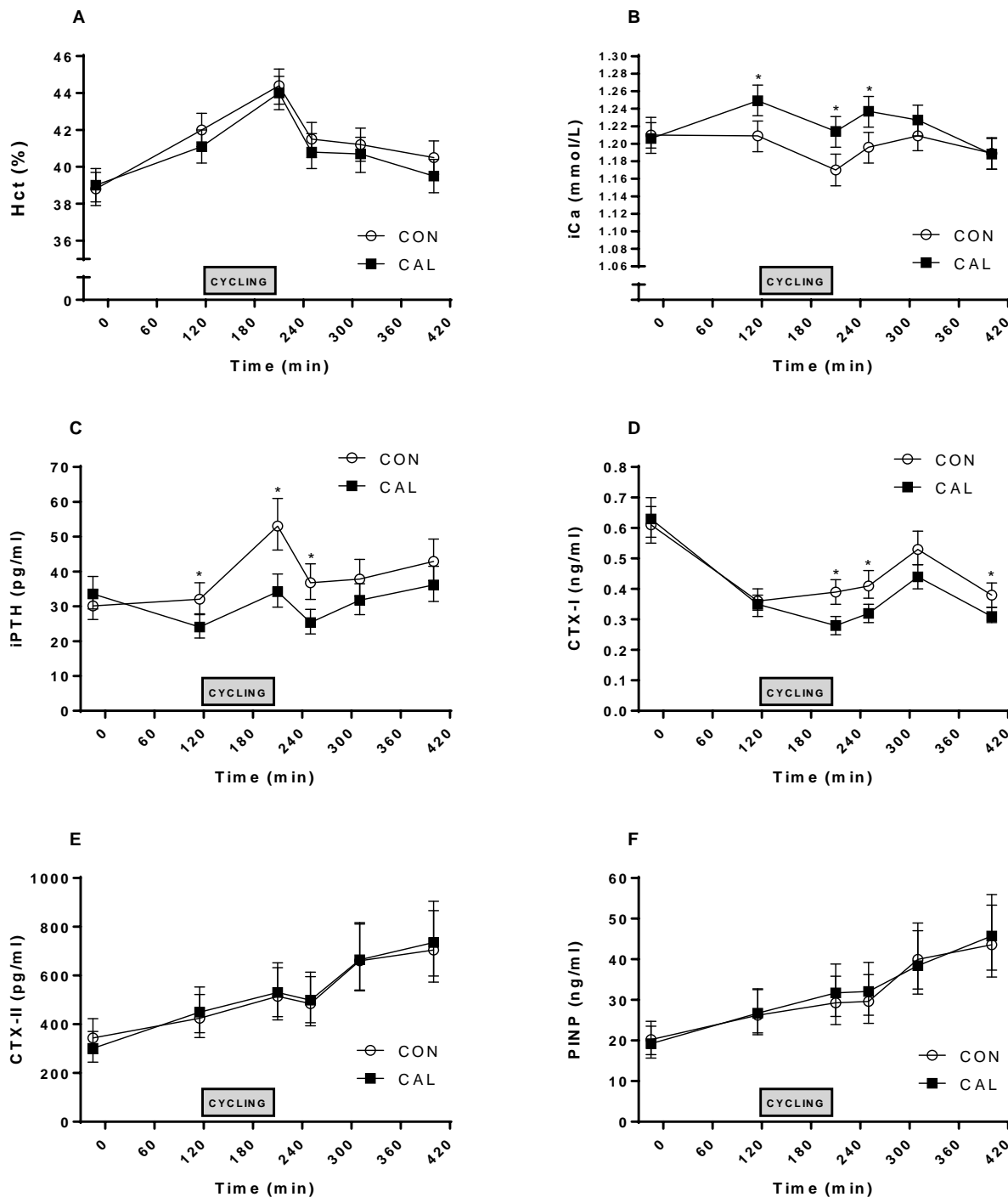


Figure 15. Serum concentrations (Mean, 95% CI) of biomarkers for bone turnover, calcium homeostasis and haematocrit before and after control and calcium-rich meal conditions and exercise

*Significant difference ($p < 0.05$) between trial meal conditions at the indicated time point Haematocrit (Hct: A); ionized calcium (iCa: B); and concentrations of *parathyroid hormone* (PTH: C); *cross linked C-telopeptide of type I collagen* (CTX-I: D); *cross linked C-telopeptide of type II collagen* (CTX-II: E); *procollagen I N-terminal propeptide* (PINP: F); and at each time point for control (CON: open circles) and calcium (CAL: solid squares) meal conditions. Blood samples were taken pre-trial at T = -15 min; pre-exercise at T = 115 min; and post-exercise at all subsequent time points.

There was a significant interaction between *pre-trial meal* and *time* for log(CTX-I) ($F_{5, 310} = 6.37$; $p < 0.01$). The median CTX-I was estimated to be 1.40 [1.15, 1.70] times higher in CON at T = 210 min, 1.30 [1.07, 1.57] times higher at T = 250 min and 1.22 [1.00, 1.48] times higher at T = 400 min ($p = 0.043$) compared to CAL. At T = 310 min the median CTX-I was estimated to be 1.20 times higher (0.99, 1.45) in CON than CAL, however the lower limit of the 95% confidence interval was 0.99 suggesting that there may not have been a difference. The interaction between pre-trial meal and time was not significant for log(CTX-II) ($F_{5, 310} = 0.56$; $p = 0.73$) or log(PINP) ($F_{5, 310} = 0.44$; $p = 0.82$). There was no effect of meal type on CTX-II ($F_{1, 31} = 0.02$; $p = 0.89$) or PINP ($F_{1, 31} = 0.29$; $p = 0.59$). The only biomarkers (iCa, PTH, CTX-I, CTX-II, PINP) that were significantly correlated were CTX-II and PINP ($R^2 = 0.86$; $p < 0.001$).

Total fluid loss was not significantly different between meal conditions (CAL: 1.21 L [1.08, 1.34]; CON: 1.17 L [1.04, 1.30]), with a mean difference of 0.04 L [-0.02, 0.01]. Sweat calcium concentration from the back was 10.3 mg/L [9.5, 11.2] for CAL and 10.4 mg/L [9.5, 11.2] for CON with a mean difference of 0.06 mg/L [-0.68, 0.80]. Bone mineral density was not significantly correlated with sweat calcium loss ($R^2 = 0.02$; $p = 0.468$), total sweat loss ($R^2 = 0.05$; $p = 0.222$) or post-exercise biomarkers of bone turnover. Bone mineral density measures are shown in Table 21. Both Z and T scores for the cyclists' Lumbar (L₁-L₄) BMD were on average below zero and seven were osteopenic (T-Score <1).

Table 21. Bone mineral density in female cyclists

	<i>Neck of Femur</i> \bar{X} [95% CI]	<i>Vertebrae L₁ - L₄</i> \bar{X} [95% CI]
<i>BMD (g·cm⁻²)</i>	1.07 [1.11, 1.02]	1.15 [1.19, 1.10]
<i>Z Score</i>	0.29 [-0.04, 0.62]	-0.26 [-0.60, 0.00]
<i>T Score</i>	0.10 [-0.23, 0.43]	-0.41 [-0.83, 0.00]
<i>T-Score < -1.0</i>	n = 3 (9%)	n = 7 (22%)

Bone mineral density (BMD); mean (\bar{X}); confidence interval (CI)

9.4 Discussion

The novel finding of this study is that the intake of a calcium-rich breakfast based on dairy sources prior to prolonged high intensity cycling attenuates the exercise-induced alterations in bone homeostasis that accompany the loss of large amounts of calcium in sweat. Specifically, a high (~1350 mg) calcium meal consumed 90 min before undertaking a 90 min bout of stationary cycling was associated with better maintained serum ionised calcium and an attenuation of the increase in markers of bone resorption (PTH and CTX-I) seen in a control trial. In contrast, markers of bone formation (PINP) were not affected by the calcium content of the pre-exercise meal. With cycling, we observed an increase in markers of resorption over ~2 h, with no change in formation markers placing the cyclist in a relatively greater resorptive state. If this occurs for several hours on most days, then bone accrual must be reduced and peak bone mass may be compromised. Elite cyclists train and compete over years in a sport with additional risk factors for poor BMD (Mountjoy et al., 2014; Scofield and Hecht, 2012). The observations in this study suggest a practical strategy that may reduce the risk of impaired bone health frequently seen among cyclists and other endurance athletes (Scofield and Hecht, 2012).

Although it sounds counter-intuitive that athletes could suffer from poor bone health given the positive association between bone loading physical activity and BMD (Milgrom et al., 2000; Turner, 1998; Turner et al., 1994), many studies report low BMD in male and female endurance athletes arising as a direct consequence of their sports participation (Scofield and Hecht, 2012). This has been attributed to low EA (Loucks et al., 2011), and is further complicated by the non-weight bearing nature of sports such as cycling and swimming (Scofield and Hecht, 2012). In cyclists, where BMD values are either low (Campion et al., 2010; Nichols et al., 2003; Olmedillas et al., 2011; Rector et al., 2008) or continuing to decrease during active participation in high level cycling (Barry and Kohrt, 2008; Lombardi et al., 2012; Sherk et al., 2014), it is of importance to identify risk factors for impaired bone health that are potentially modifiable. This has created interest in the role of increased bone resorption secondary to acute changes in serum ionic calcium concentrations arising from the dermal loss of calcium through sweat (Barry and Kohrt, 2008; Barry et al., 2011; Guillemant et al., 2004). According to the limited available literature, there are indications of an increase in bone resorption during exercise involving significant sweat calcium loss, with an apparent attenuation of that negative outcome when calcium supplements are consumed before or during exercise (Barry et al., 2011; Guillemant et al., 2004). In one

investigation, the intake of calcium fortified (1000 mg) water prior to and during exercise was considered to reduce the potential perturbations to bone calcium, as seen by an attenuation of the significant increase in PTH associated with 60 min of cycling. In this study, the exercise-associated changes in CTX-I during and immediately after exercise were not significantly different as a result of calcium intake (Barry et al., 2011). Meanwhile, Guillemant et al. (2004) found that 30 min following completion of ~60 min of stationary cycling, the rise in CTX-I was lower as a result of calcium supplementation. Our study supports and extends these findings.

Our results provide evidence that markers of bone resorption can remain elevated for approximately 2 h following 60 - 90 min of cycling. Furthermore, we show that a high calcium meal sustains attenuation of the rise in CTX-I seen with exercise, with clear reductions in blood concentrations immediately and 40 min following a 90 min bout of cycling. Although CTX-II was originally proposed to be a marker of cartilage degradation (Christgau et al., 2001), recent evidence suggests it is more strongly correlated with other markers of bone resorption (van Spil et al., 2013) and it therefore might have been expected to mirror the movements of CTX-I. In contrast, CTX-II was not affected by the pre-exercise meal in our study, but was strongly correlated with PINP, a proposed marker of bone formation. We established a stronger relationship than has been previously reported (Christgau et al., 2001), which suggests that further investigations into what aspect of bone or cartilage turnover CTX-II represents, may be warranted. Differences between our results and those from previous investigations may be attributed to differences in duration and intensity of the exercise protocols previously used and differences in the time course of blood sampling. In the present study we did not sample blood during exercise; this needs to be considered when interpreting the results. However, given that Guillemant et al. (2004) showed a somewhat linear increase in PTH and CTX-I during exercise it was believed that blood sampled prior to and at several time-points following exercise would be sufficient to capture exercise-induced changes in these variables and the effects of meal type.

The premise and design of our study was based on general recommendations for calcium intake for bone health as well as the specific results of two earlier investigations on calcium intake in relation to exercise (Barry et al., 2011; Guillemant et al., 2004). An intake of 1000 mg of calcium per day has been indicated through meta-analysis as a threshold intake required to confer beneficial effects on BMD in combination with physical

activity (Specker, 1996). Similarly, 1000 mg is described as an adequate daily intake for males and females older than 19 y in the Dietary Guidelines for Australian Adults (2013). It has been suggested that a daily intake of 1500 mg would be required by male cyclists to maintain a positive calcium balance after accounting for absorption rates and sweat losses (Barry and Kohrt, 2008). Studies utilising a calcium fortified beverage specifically around a cycling bout provided a 1000 mg calcium dose, with cyclists consuming this immediately before and/or during the exercise session (Barry et al., 2011; Guillemant et al., 2004). However, this strategy requires athletes to consume 1 L of fluid, 20 - 60 min before exercise and 250 ml every 15 min during, which is substantially more than athletes would typically be advised to consume (Kenney, 2004; Sawka et al., 2007). The meal utilised in this study was designed to include >1300 mg of calcium to ensure sufficient biologically-available dietary calcium in the pre-exercise meal. We found that it confers benefits to bone metabolism similar to calcium fortified water, does not impair gut comfort or performance (Haakonssen et al., 2014b) and may be more practical to integrate into the athlete's daily routine. Further work may be necessary to establish more accurately how the source, amount and timing of calcium intake interact. The necessary threshold for the described effects may be lower and may have individual variability.

Future studies should use a higher frequency of blood sampling to more accurately determine the duration at which markers of bone resorption cease to increase. Using exercise protocols with a longer duration that more accurately reflects an elite cyclist's training load may also affect the time taken for markers of bone resorption to return to baseline. A typical training week for an elite cyclist often exceeds 20 h on the bike, with individual training sessions lasting anywhere between ~2 – 6 h. It is of interest to establish whether the findings from studies using comparatively short bouts of cycling (<90 min) can be extrapolated to longer exercise periods with potentially larger dermal calcium losses. If so, this factor may well contribute to the well documented reduction of BMD reported for male and female cyclists but have a potential solution to reduce its impact. Additional work should also consider the bone turnover effects of high sweat calcium losses that occur independent of exercise or large losses that occur in conjunction with weight-bearing physical activity that is bone loading. For example, O'Toole et al. (2000) showed that fire fighter recruits had no changes in BMD after 4 mo of training which included frequent bouts of high work rates lasting 3 - 4 h and resulting in substantial fluid losses of ~2.5 L; more than double the loss observed in the current study. It is of interest to ascertain

whether weight-bearing activities are able to offset the potential effect of dermal calcium losses on bone turnover.

We also failed to find a correlation between the magnitude of exercise-induced sweat calcium losses, absolute and relative measures of biomarkers of bone turnover and BMD in the current cohort of subjects. This is in contrast to the observations of Barry and Kohrt (2008) who found an inverse relationship between their estimates of dermal calcium losses and BMD in a group of male cyclists. It should be noted that these cyclists were on average 10 y older and possibly had a longer training and racing history than our subjects, potentially making a relationship between sweat calcium loss and BMD easier to identify. However, given the complex interplay between mechanical loading, nutrition and endocrine function, it could be expected that calcium sweat concentration would explain only a small proportion of the variance in BMD and indeed change in BMD over time. Clearly, long-term, prospective studies are still required to determine whether pre-exercise calcium intake (dairy or supplement) can attenuate long term reductions in BMD previously described in cyclists, particularly when other factors that contribute to bone health are difficult to alter. One known study (Mathis-Korgaokar et al., In Press) found no difference in BMD when a calcium carbonate supplement was consumed by cyclists prior to exercise for a 5 mo period. Given the subtle changes in BMD that occur over the competitive season (1.5%; Barry and Kohrt, 2008), longer periods (>1 y) of observation may be required.

Finally, the dairy-based pre-exercise meal used as the intervention in this study, may help athletes to achieve a number of guidelines related to health and sports nutrition goals. An increase in dairy foods in the general diet is a key recommendation of population dietary guidelines, with the recommended dietary intake (RDI) of dairy in Australia for females aged 18 - 60 y being 2.5 serves per day (Australian Dietary Guidelines, 2013). As well as the importance of dairy-derived calcium to bone health, dairy intake has been promoted, albeit controversially (Lanou and Barnard, 2008), as having a beneficial effect on body composition (Phillips and Zemel, 2011; Zemel, 2004; Zemel et al., 2004). Indeed, consuming a high calcium diet in a hypoenergetic state has been shown to accelerate weight loss, with augmented effects and a greater proportion of body fat loss when dairy is the calcium source (Zemel et al., 2004). This may be important for cyclists for whom producing a relatively high power output relies on having a low BW while maintaining sufficient functional lean mass. Guidelines for protein intake to enhance muscle protein

synthesis now recommend an even spread of high quality protein sources every ~3 h over the day, and it is noted that typical food choices included in Western breakfast patterns are unlikely to meet the target of 20 - 25 g of protein per meal (Areta et al., 2013). This is even more likely if dairy foods are excluded from breakfast/pre-training choices. Our dairy-rich meal provided ~36 g of protein (based on a 60 kg athlete) including a rich source of whey protein which is particularly valuable for muscle protein synthetic needs (Phillips and Zemel, 2011).

9.4.1 Conclusion

While the interest in nutrition and training with elite athletes is most commonly focused directly on performance, bone health has implications for long term function and may also reduce the risk of fracture; a common outcome of accidents in road cycling races. The present study shows that a high-calcium dairy based pre-exercise meal can reduce the exercise-induced rise in markers of bone resorption. This practice can easily be incorporated into the dietary routine of endurance cyclists and may help the athlete meet other sports nutrition recommendations. Future studies should use a long-term prospective design to confirm the efficacy of pre-exercise calcium intake for improving bone health in endurance cyclists.

CHAPTER 10: SUMMARY AND CONCLUSIONS

The general aim of the present research was to investigate factors relating body composition to performance and health in elite female road cyclists. Potential strategies were examined that may assist in making the manipulation and maintenance of physiques that perform effective, healthy, sustainable and practical. Ensuring that these criteria were addressed and providing clear, practical recommendations that could be applied in the context of high performance road cycling was a key objective. The first study (Chapter 3) sought simply to establish the perceptions that female road cyclists had towards body weight (BW) as it relates to performance. Questionnaire data collected from a representative sample of elite female cyclists at the Australian National Road Cycling Championships confirmed that a majority of road cyclists participating in the study were dissatisfied with their BW in the lead up to this major competition. The findings were affected by the cyclists' specialisation, as flat-land specialists reported a greater difference between their current and ideal weight compared to climbers. This suggests that weight satisfaction may be course specific when framed in the context of a specific race. It is noteworthy that 73% of cyclists surveyed reported that their career lowest weight was beneficial for performance and almost all cyclists reported that they agree; *female cyclists are a weight conscious population*. These findings suggest that the population is acutely aware of the benefits of being light, particularly for climbing performance. The study also identified that some of the strategies used by athletes to reduce BW might be detrimental to health, and a number of the cyclists reported having been diagnosed as having an eating disorder. Caution should therefore be taken with the way sports scientists and coaches communicate and implement strategies aimed at manipulating or maintaining body composition. One major limitation was that the questionnaire used in this study has not been assessed for validity or reliability. It is however, novel, in that it relates questions about body composition specifically to performance in the upcoming race. No other known questionnaires have sought responses to population-specific questions. Future studies should address these limitations and further investigate the health implications of participating in the sport, particularly in relation to menstrual status and other measures of relative energy deficiency.

The second study (Chapter 4) described estimates of body composition of female cyclists categorised as sub-elite, elite or world class. The findings confirmed those from previous

research and showed that world class female cyclists were lighter and leaner than cyclists performing at the lower levels; a clear relationship between body composition and performance was found. It is likely that a host of physiological adaptations contribute to the progression and success of female cyclists. Studies attempting to identify direct relationships between body composition and performance will face confounding factors associated with these training adaptations. As an athlete increases their training load, body composition changes will occur that might contribute to performance but so too will other adaptations. Nonetheless, the findings from this investigation show an association between race performance and body composition in a large sample of riders who were tracked over a 16 y period. These data provide normative values for world class female road and track endurance cyclists which may guide athletes and sport scientists towards realistically achievable goals. It was further shown that BW, and particularly skinfolds, fluctuate within a season; measures are higher in the off-season than during the competitive season. As a general practice, the sport scientist should collect anthropometric data more frequently in order to determine biological variability, as well as changes attributed to training, dietary interventions and extended periods of racing. The data set assessed in the second study is observational and does not indicate the true magnitude of achievable change.

The third study (Chapter 5) attempted to make direct comparisons between body composition and performance outcomes in female cyclists. Rather than focusing on total BW and fat mass, it measures the potential relationship between functional lean mass (lower body lean mass: LBLM) on maximal power output measured over durations ranging from 1 s through to 30 min. The results showed moderate-to-strong correlations between functional lean mass and power output, although the relative contribution of lean mass reduced as the duration of the cycling effort increased. This suggests that lower body lean mass is particularly important for short maximal accelerations or sprints which are common in road cycling (e.g. attacks and sprints). Limitations to the methodology include the effect of pedalling rate (cadence) as a possible confounder to the maximal power outputs measured in the shorter (<6 s) efforts. Future studies should use inertial load testing to identify both maximal power output and optimal pedalling rate as a surrogate for fibre type as described by Martin et al. (2000). Nonetheless, the findings from this study provide normative data for female cyclists, using a novel approach that describes muscle quality in a way that can guide training methodology. They also emphasise the importance of using

strategies that preserve lean mass during periods of energy restriction with the objective of reducing BW.

Given that exercise accounts for the largest component of total daily energy expenditure (EE) with endurance athletes, accurate measurement of EE is important for guiding interventions in which a determination of energy balance (EB) or energy availability (EA) is required. In the fourth study (Chapter 6), the use of power meters to estimate EE during variable intensity cycling, were validated against indirect calorimetry. Gross efficiency (GE) was measured using an incremental sub-maximal stationary cycling protocol before and after a ~10 min bout of variable intensity cycling that mimicked the power demands of a typical road ride. The regression model for power output and GE allowed for power based estimates of EE during variable intensity cycling that were in all cases within 3% of estimates made using indirect calorimetry. These results show that the power meter is a valid tool for estimating EE during variable intensity cycling at intensities less than 75% of the athlete's maximal aerobic power (MAP).

In study five (Chapter 7), a case was presented in which a female cyclist undertook a retraining intervention with a strong focus on body composition manipulation. Using DXA and anthropometry to measure body composition, this case study showed that despite a subtle energy restriction that elicited substantial fat loss, lean mass was increased and training adaptations promoted. These favourable outcomes were attributed to an intervention that combined strength-based training (gym and bike) with a nutrition plan that placed the greatest energy intake (EI) around training and increased the consumption of high biological value proteins such as dairy and lean meats. The results suggest that despite being energy restricted, the athlete was in an anabolic state. Future interventions of this nature should employ the more accurate GE protocol described in study four (Chapter 6). A controlled study with a sufficient sample is required to support the reasoning proposed in this study.

As discussed at length, female cyclists compete in a weight-sensitive sport and are at risk of relative energy deficiency and the associated poor health outcomes. In studies six (Chapter 8) and seven (Chapter 9), the effects of a calcium-rich pre-exercise meal on gut comfort, cycling time trial performance and biomarkers of bone resorption were investigated. Prior to assessing the efficacy of a health-based intervention with athletes, it is important to ascertain whether it can be practically implemented in the daily training

environment without detriment to performance. In the athlete's mind, performance is paramount and if this is impaired, compliance to a proposed strategy may well be compromised. The results from study six showed that a calcium-rich pre-exercise breakfast meal could be consumed without impairing performance or causing gut discomfort any more so than a control meal. Further, there was no statistically significant difference in the palatability reported for the two meals. A limitation of this study was the lack of a validated questionnaire for gut comfort as well as the use of stationary cycling for only 90 min. In reality, training sessions and races will often last several hours and take place on the road where the intensity is much more variable. In addition, the 10 min time trial is not a validated performance test although it was impractical to utilise a longer protocol within the time frame available. It is acknowledged that the implementation of the questionnaire at the end of the 80 min pre-load (55% maximal aerobic power output), just prior to the final 10 min time trial, may not have identified differences in gut comfort. Furthermore, the study did not control for previous dairy or calcium consumption. It is possible that the study attracted a disproportionate number of cyclists who typically eat dairy and therefore, the sample may not have been representative of the population. While this is possible, the sample was relatively large in comparison to the population and several lactose intolerant athletes did express interest in participating. The researchers therefore believe that the sample was largely representative.

Study seven (Chapter 9) examined the effects of a calcium-rich dairy meal on biomarkers of bone turnover. It was found that following 90 min of stationary cycling, biomarkers of bone resorption were higher when the control meal was consumed compared to the calcium-rich meal. At the same time, there was no difference in biomarkers of bone formation between the two conditions. Female cyclists perform training sessions that last up to 6 h, where they could be expected to experience substantial dermal calcium losses through sweat. If 90 min of cycling is sufficient to disturb calcium homeostasis and increase bone resorption, it could be expected that this disturbance is magnified or at least prolonged in response to training of longer duration. The consumption of a dairy based meal appears to attenuate this disturbance. As it is well established that female cyclists have a high prevalence of low bone density and have a number of risk factors that might accelerate this, any strategy that might mitigate the risk should be encouraged. Incorporating a calcium-rich dairy based meal in the form of breakfast prior to training is something that cyclists can practically integrate into the daily diet and training regimen, without impairing performance in subsequent training or racing. Introducing dietary

calcium through a dairy meal may be more beneficial than consuming calcium-fortified water as it may assist athletes in meeting general recommendations for calcium intake and athlete specific recommendations for intake of high biological value proteins that stimulate muscle protein synthesis. Limitations to the study included the use of a relatively short period of exercise and a low frequency of blood sampling for the purpose of measuring biomarkers. Future studies should address these limitations to better elucidate the time response of these biomarkers to exercise. It is also noteworthy that direct causality between sweat calcium loss and poor bone health has not yet been fully established. It may be possible that the elevated bone resorption during exercise is part of the normal bone remodelling process. Long term (>12 mo) prospective studies are required to confirm the efficacy of a pre-exercise calcium intake for attenuating reductions in bone density. The evidence from these last two studies, in combination with previous work, does however suggest that increasing pre-exercise calcium intake is warranted. The proposed dairy meal presents as a logistically simple method for doing so, while also helping athletes meeting other nutritional goals.

In conclusion, the investigations described in this thesis add substantially to the limited research that has investigated the interactions between body composition, performance and bone health in female cyclists. Female cyclists certainly appear to be a weight conscious population. Over the last 16 y there has been a clear performance relationship, where world class female cyclists are lighter and leaner than lower performing cyclists. While there is a relationship between performance and low body fat / low BW, there is also evidence that functional muscle mass should be preserved to optimise power producing capacity. There is evidence that even in the presence of subtle energy restriction, high biological value protein coupled with strength based training may offer stimuli that facilitate the reduction of fat mass while simultaneously increasing protein synthesis. Strategies aimed at manipulating body composition, should use valid, reliable tools to quantify EE and EI. When calibrated, the SRM power meter can be used as an accurate tool for estimating EE relative to EI. Any intervention performed with an elite athlete should prioritise their health. There is reasonable evidence to suggest that consuming a calcium-rich pre-exercise meal attenuates bone resorption which occurs in response to exercise.

There is still much to be studied in this broad area of body composition, as it has complicated interactions with training, diet, psychology, health and performance. Future studies should investigate the efficacy of various diet and training interventions for the

specific purpose of manipulating body composition using controlled study designs. Body composition modification is a desirable training adaptation in road cyclists and there remains little research which is relevant to the development of best practices.

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APPENDIX A: FEMALE CYCLIST WEIGHT MANAGEMENT QUESTIONNAIRE

Female Cyclist Weight Management Questionnaire

1. Do you race for a professional team competing internationally Yes / No (circle one)
 If Yes: For how many years? (check one box)
 1 - 2 3 - 4 5 or more
2. What was your best ranking in a National Racing Series (NRS) race between 2012 and today? (check one box)
 Top 3 4 - 10 11 - 20 Never top 20 I have never raced in the NRS Didn't race NRS because I was racing overseas
3. What type of a cyclist would you say you are? (check one box)
 Climber Sprinter Time Trialist All rounder (rouler)
4. What is your height? _____ cm / ft.in
5. What is your CURRENT body weight? _____ kg / lb
6. What is the LOWEST body weight you have competed at since you stopped growing? _____ kg / lb
7. What is the HIGHEST body weight you have competed at? _____ kg / lb
8. Do you think your current body weight is optimal for performance at this race? Yes / No
 If No: What would be your IDEAL body weight? _____ kg / lb
9. How do you think being at your LOWEST weight affects your race performance? (check one box)
 Extremely Harmful Somewhat Harmful No Effect Beneficial Extremely Beneficial
10. Has a doctor ever diagnosed you with an eating disorder? Yes / No
11. Do you believe female cyclists are a weight conscious population? Yes / No
12. Has your coach / director expressed dissatisfaction with your body weight in the past 12 months? Yes / No

Subject Number _____

13. Have you tried to change your body weight in the last 12 months? Yes / No
 If No: continue to Question 13
 If Yes: Did you try to increase or decrease your body weight? (circle one)
 Were you successful at changing your body weight? Yes / No
 How much weight did your weight increase or decrease? _____ kg / lb
14. In the last 12 months, how often were you conscious about wanting to change your body weight? (circle one number)
 Never Once in the year More than once a year Once a month More than once a month Once a week More than once a week Once a day More than once a day Every hour More than every hour
 0 1 2 3 4 5 6 7 8 9 10
15. Have you ever used any of the following techniques for the specific purpose of reducing body weight? (check as many as apply)
 I reduced food intake throughout the day I avoided foods high in fat
 I increased daily training duration I avoided foods high in sugar
 I wore additional clothes or plastic wraps when training I skipped breakfast, lunch or dinner
 I performed long training rides (>2hrs) without eating I avoided eating after training
 I used weight loss supplements or medications I made myself vomit after eating
 Describe others: _____ None of the above
16. Do you still menstruate (have your period)? Yes / No
17. Do you use an oral contraceptive pill, intrauterine device (IUD) or implant which prevents regular menstruation? Yes / No
18. Have your periods stopped for more than 3 months during the last year without you being pregnant? Yes / No
19. Have your periods been regular during the last year? Yes / No / Unsure
 'Regular' means the periods lasted about as long each time with about the same time between them.

APPENDIX B: SERUM CONCENTRATIONS OF BIOMARKERS FOR BONE TURN OVER, CALCIUM HOMEOSTASIS AND HAEMATOCRIT BEFORE AND AFTER CONTROL AND CALCIUM-RICH MEAL CONDITIONS AND EXERCISE

<i>Biomarker</i>	<i>Time (min)</i>	<i>CON</i>		<i>CAL</i>	
		\bar{X}	[95% CI]	\bar{X}	[95% CI]
<i>Hct (%)</i>	-15	38.8	[37.9, 39.7]	39.0	[38.1, 39.9]
	115	42.0	[41.1, 43.0]	41.1	[40.2, 42.0]
	210	44.4	[43.4, 45.3]	44.0	[43.1, 44.9]
	250	41.5	[40.5, 42.4]	40.8	[39.9, 41.8]
	310	41.2	[40.3, 42.1]	40.7	[39.7, 41.6]
	400	40.5	[39.6, 41.4]	39.5	[38.6, 40.4]
	<i>Unadjusted iCa (pg/ml)</i>	-15	1.21	[1.19, 1.23]	1.21
115		1.21	[1.19, 1.23]	1.25	[1.23, 1.27]*
210		1.17	[1.15, 1.19]	1.21	[1.20, 1.23]*
250		1.20	[1.18, 1.21]	1.24	[1.22, 1.25]*
310		1.21	[1.19, 1.23]	1.23	[1.21, 1.24]
400		1.19	[1.17, 1.21]	1.19	[1.17, 1.21]
<i>iCa (pg/ml)</i>		-15	1.21	[1.18, 1.25]	1.21
	115	1.21	[1.18, 1.24]	1.25	[1.22, 1.28]
	210	1.10	[1.07, 1.13]	1.13	[1.09, 1.16]
	250	1.21	[1.18, 1.24]	1.25	[1.21, 1.28]
	310	1.23	[1.20, 1.26]	1.24	[1.21, 1.27]
	400	1.23	[1.20, 1.27]	1.20	[1.17, 1.23]
	<i>PTH (pg/ml)</i>	-15	30.11	[26.20, 34.60]	33.60
115		32.03	[27.87, 36.80]	24.04	[20.92, 27.63]*
210		53.05	[46.16, 60.96]	34.19	[29.75, 39.28]*
250		36.75	[31.99, 42.23]	25.37	[22.08, 29.16]*
310		37.85	[32.94, 43.50]	31.74	[27.62, 36.47]
400		42.93	[37.36, 49.33]	36.11	[31.43, 41.49]
<i>CTX-I (ng/ml)</i>		-15	0.61	[0.55, 0.67]	0.63
	115	0.36	[0.33, 0.40]	0.35	[0.31, 0.38]
	210	0.39	[0.35, 0.43]	0.28	[0.25, 0.31]*
	250	0.41	[0.37, 0.46]	0.32	[0.29, 0.35]*
	310	0.53	[0.48, 0.59]	0.44	[0.40, 0.49]
	400	0.38	[0.34, 0.42]	0.31	[0.28, 0.34]*

Appendix B continued

<i>Biomarker</i>	<i>Time (min)</i>	<i>CON</i>		<i>CAL</i>	
		\bar{X}	[95% CI]	\bar{X}	[95% CI]
<i>CTX-II (pg/ml)</i>	-15	343.94	[279.70, 422.94]	300.91	[244.70, 370.02]
	115	424.39	[345.13, 521.86]	449.77	[365.77, 553.07]
	210	514.24	[418.19, 632.35]	530.18	[431.16, 651.95]
	250	484.47	[393.98, 595.74]	499.05	[405.84, 613.67]
	310	660.03	[536.75, 811.62]	664.38	[540.29, 816.97]
	400	704.72	[573.09, 866.57]	735.40	[598.05, 904.31]
	<i>PINP (ng/ml)</i>	-15	20.21	[16.52, 24.73]	18.21
115		26.20	[21.42, 32.50]	26.74	[21.86, 32.71]
210		29.29	[23.94, 35.83]	31.73	[25.94, 38.82]
250		29.62	[24.22, 36.24]	32.07	[26.22, 39.24]
310		39.97	[32.67, 48.90]	38.43	[31.41, 47.01]
400		43.60	[35.64, 53.35]	45.70	[37.35, 55.91]

Control meal condition (CON); calcium rich meal condition (CAL); haematocrit (Hct); Ionized calcium (iCa); parathyroid hormone (PTH); cross linked C-telopeptide of type I collagen (CTX-I); cross linked C-telopeptide of type II collagen (CTX-II); procollagen I N-terminal propeptide (PINP); Mean (\bar{X}); confidence interval (CI).

*Significant difference ($p < 0.05$)