University of Alberta

Energy availability of female athletes and non-athletes taking oral contraceptive pills

by

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This thesis is dedicated to all athletes who devote their time, resources, and health to pursuing physical excellence.

Excellence is an art won by training and habitation. We do not act rightly because we have virtue or excellence, but we rather have those because we have acted rightly.
We are what we repeatedly do.
Excellence, then, is not an act but a habit.

-Aristotle-

ABSTRACT

The purpose of this study was to estimate energy availability (EA) in healthy females taking oral contraceptive pills (OCP). Participants (n=12 athletes and n=11 non-athletes) completed 2 separate 7-day dietary intake and exercise energy expenditure (EEE) logs across two consecutive pill cycles. Recording started between days 1 - 4 of the individual's pill cycle. Resting energy expenditure was measured and used to correct MET values to determine EEE. The groups were similar in age, weight, BMI, age of menarche, and gynecological age. Athletes had significantly higher fat free mass and lower percent body fat ($p<0.001$). Their EA (33 and 31 kcal/kg FFM/day week 1 and 2, respectively) was statistically lower ($p<0.001$) than the non-athlete group (49 and 47 kcal/kg FFM/day week 1 and 2, respectively). Compared to the EA weight maintenance recommendations reported in the literature, EA values of these athletes are lower.

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'If you want to go quickly, go alone, if you want to go far, go together' Apache saying

TABLE OF CONTENTS

APPENDICES

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AA – Amenorrheic athlete
ACSM – American College of Sport Medicine
BMD – Body mineral density
BMI – Body mass index
CHO - Carbohydrate
CVD – Cardiovascular disease
DEBQ-R – Dutch Eating Behavior Questionnaire – Restraint Scale
DLW – Doubly labeled water
DRI – Dietary reference intakes
DXA – Dual energy x-ray absorptiometry
EA – Energy availability
EDI – Eating disorder inventory questionnaire
EE – Ethinylestradiol
EEE – Exercise energy expenditure
EPOC – Post exercise oxidative consumption
FFM – Fat free mass
FHA – Functional hypothalamic amenorrhea
FSH – Follicle stimulating hormone
GnRH – Gonadotropin releasing hormone
HPA – hypothalamic pituitary adrenal axis
HR – Heart rate
IOC – International Olympic committee
LBM – Lean body mass
LH – Luteinizing hormone
LPD – Luteal phase defects
MET – Metabolic equivalents
PAR-Q – Physical activity readiness questionnaire
PdG – Peak progesterone
OCP – Oral contraceptive pill
OC – Oral contraception
REE – Resting energy expenditure
SHBG – Sex hormone binding globulins
TDEE – Total daily energy expenditure
VO_2max – Maximal volume of oxygen

UNITS

kcal – kilocalorie
kcal/day – kilocalorie per day
kcal/kg FFM/day – kilocalorie per kilogram fat free mass per day
IU – international units
mg - milligram
ml/kg/min – millilitre per kilogram per minute
pg/ml – picogram per millilitre
pmol/l – picomoles per litre
ug/ml – microgram per millilitr

CHAPTER 1
INTRODUCTION

Justification of Study

Research surrounding energy availability (EA) and menstrual dysfunction has grown to include other factors for consideration (i.e. gynecological age, definition of an athlete, menstrual status classification confirmed with hormonal markers, psychogenic stress, oral contraceptive use), potentially allowing for more tailored nutrition recommendations for the individual female athlete. However, the EA values providing the premise for the recommendations have been conducted under controlled laboratory settings and the transferability to exercising females in the natural settings of the athlete, including athletes taking oral contraceptive pills (OCP), is limited. Currently this research has excluded females taking OCP, as they do not follow a normal menstrual pattern. However, with the increase prevalence of OCP use in athletics, it is necessary to examine the effect OCP has on EA in future studies. This research will contribute to improved nutrition prescription of female athletes to maintain health and pursue athletic excellence. The constraints of measuring menstrual status, energy intake and energy expenditure in an exercising setting can be overcome to develop a framework for successfully managing training programs and the health of female athletes.

Purpose

Exercise-associated menstrual cycle disturbances are linked to an array of clinical consequences including decreased bone mineral density, increased incidence of stress fractures[1], reduced recovery from exhaustive exercise [2,3], and endothelial dysfunction.[4] In a series of well-controlled studies it has been demonstrated that these reproductive disruptions result from a low EA and that exercise by itself, apart from the cost on EA, has no disruptive effect.[5,6] EA is defined as

dietary energy intake minus exercise energy expenditure. These studies have identified an EA threshold of 30 kcal/kg fat free mass (FFM)/day, below which reproductive markers are altered.[7] As most of these findings have been determined in sedentary women, it is surprising that the same EA thresholds have been recommended for female athletes.[8,9,10] To date the relationship between reproductive disruptions and the EA threshold has not been examined in trained females. Research in this area has been lacking due to the burden of collecting dietary intake and energy expenditure over long periods of time combined with invasive menstrual cycle monitoring procedures. In addition, female athletes using OCP have largely been excluded from the research, even considering the prevalence of their use. Therefore, the purpose of this study is to estimate the EA of female athletes and non-athlete taking OCP using short-term noninvasive tools.

Hypothesis

Healthy, non-athlete age-matched females taking OCP will have EA values (measured as kcal/kg FFM/day) greater than female athletes taking OCP.

Delimitations

The 23 participants included in this study were females between the ages of 20 – 32 yrs and met all the inclusion and exclusion criteria outlined in the Methods section.

Limitations

Self-Report

This study relied on a variety of self-reporting procedures. Inclusion into the study relied on self-report of medical and demographic history. Throughout the study, participants recorded energy intake and exercise energy expenditure through self-report logs. It was assumed that all participants answered the questions and completed the records with honesty.

Participant Compliance

Adherence to the protocol for the OCP regime was not under researcher control, however in light of the reasons for OCP use, it was assumed that the participants would adhere to the routine to prevent unwanted pregnancy, control of premenstrual symptoms, and/or cycle consistency. Accuracy of recording intake and exercise was also left to the discretion of the participants, although the researcher provided as much detail and assistance to ensure accurate information was recorded. There was also a 2-month commitment to the study and motivation to continue with the protocol might have waned. Throughout the study the participants could have made the decision to not continue using OCP, ultimately the participant's decision, and therefore would have been required to withdraw from the study. This was not the case and all participants completed the study.

Sample Size

To calculate sample size to attain at least a power of 80%, EA values from previous research were used. De Souza et al (1998) calculated EA on sedentary ovulating subjects and exercising ovulating females using a 7-day food record and activity logs as 30.0 ± 1.2 and 23.3 ± 1.6 kcal/kg BW/day, respectively.[11] The body mass unit to describe the relative EA value was kg of total body weight. Since lean body mass (LBM) was provided in the paper EA values were recalculated, resulting in an EA for the sedentary group of ~ 38 kcal/kg LBM/day and 32 kcal/kg LBM/day for the exercising group.[11] De Souza et al, had 0 kcal/day exercise expenditure for the sedentary group. The control group in the present study will likely have some values for exercise expenditure and therefore a projected 100 kcal/day was used to adjust the EA to be more representative of the sedentary group. The projected mean for the non-athlete and athlete groups were 35 and 32 kcal/LBM/day, respectively, with a significance level of 0.05 and a SEM of 2.0

for both groups. A specific power calculator was used based on these values to predict sample size.[13] Using the above values a projected sample size of n=13 for both groups gave a power of 95%.

Basic Menstrual Cycle Physiology

The hypothalamus, pituitary, and ovaries 'communicate' through an intricate process to ensure a female undergoes appropriate preparation for conception. Hormone secretions direct the communication and regulate the process through feedback mechanisms. The gonadotropin releasing hormone (GnRH) pulse generator is a network of some 1000 neurons located in the hypothalamus that coordinate the release of GnRH in a pulsatile fashion.[14] GnRH travels through the portal vessels to the anterior pituitary gland where it binds to its receptors to stimulate the release of the gonadotropins, luteinizing hormone (LH) and follicular stimulating hormone (FSH). These hormones are released systemically, traveling through the blood to reach their target receptors in the ovaries to stimulate the release of steroidal hormones. In females the pattern of LH and FSH secretion varies throughout the menstrual cycle based on both positive and negative feedback control from the ovarian hormones, estradiol and progesterone. These ovarian hormones can act at the level of the hypothalamus by modulating GnRH pulse frequency and by altering the ability of GnRH to stimulate LH and FSH secretion from the pituitary.[17] Estradiol can be stimulatory or inhibitory on the GnRH pulse generator differing from progesterone, which is inhibitory.[9] Other inhibitory and stimulatory signals on the GnRH pulse generator exist. This is beyond the scope of this review; the reader is referred to Tsutsumi et al, Fernandez-Fernandez et al, and Meczekalski et al.[14,15,16]

The pulsatile release of GnRH is the essential component governing reproductive function.[14] The pulsatile characteristic of GnRH secretion, both the amplitude and frequency,

plays a key role in the maintenance of menstrual function.[14] One reason includes regulation of GnRH receptors in the pituitary. Another reason is that the synthesis and secretion of LH and FSH are regulated and maintained by the frequency of the GnRH pulses at the hypothalamus level. Changes in the frequency of the pulses favors the secretion of either LH or FSH creating an appropriately matched reaction to the alteration in the steroidal hormone level.[14]

The menstrual cycle is distinguished by two phases separated by ovulation, the follicular and luteal phases. During the early follicular phase, FSH is dominant and stimulates maturation of the developing follicle. The granulosa cells of the developing follicle produce and secrete estrogen. The increasing rise in estrogen in the mid follicular phase stimulates GnRH pulse frequencies to increase, favoring greater LH synthesis and secretion of low amplitude, high frequency pulses (1 pulse every 90 minutes). The surge of LH stimulates ovulation, starting 34 – 36 hours before and peaking 12 – 24 hours before.[17] Following ovulation, early luteal phase, the maturation of the follicle transforms into the corpus luteum, which continues to secrete estrogen and initiates progesterone secretion. After ovulation progesterone production predominates and elicits a slowing of the GnRH pulse secretion that in turn suppresses the frequency and increases the amplitude of LH pulses, decreasing LH concentration and favoring FSH production. GnRH and LH pulse frequency is reduced to 1 pulse every 3-4 hours.[17] If fertilization does not occur following ovulation, luteolysis (regression of the corpus luteum) is initiated, marking a rapid decline in progesterone levels. The uterine lining disintegrates and sloughs away initiating a menstrual flow. Day 1 of the menstrual cycle is defined as the start of this shedding of the uterus lining. There is now no inhibiting steroidal hormone signal on FSH and LH and the process repeats.[17] Figure 1.0 summarizes this description of the menstrual cycle.

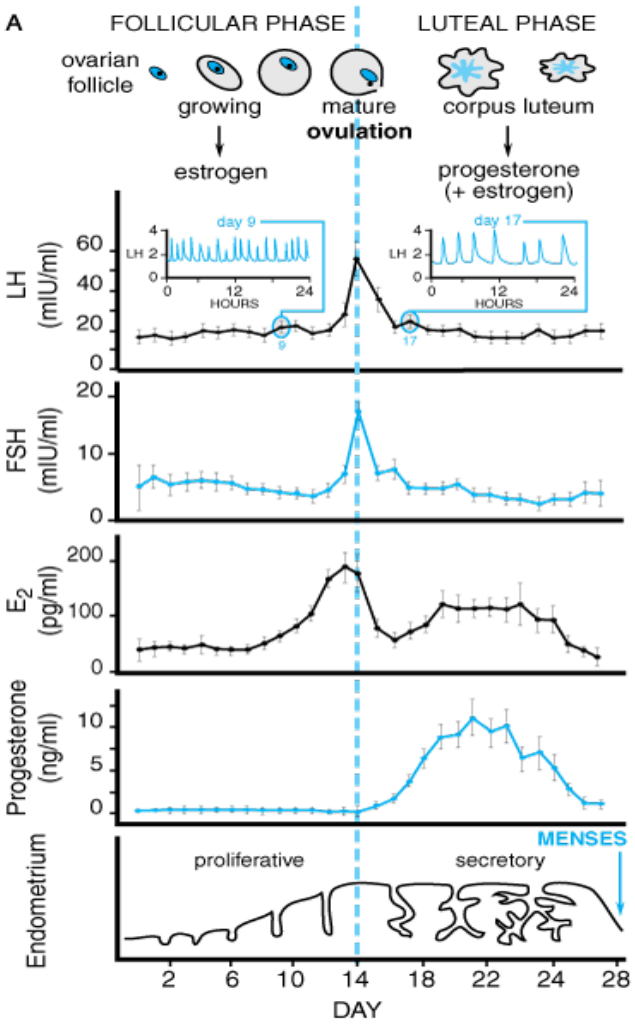


Figure 1.0 The Female menstrual cycle

The length of the above process is defined as 1 menstrual cycle, from the onset of menstrual bleeding until the day before the next bleeding. This time frame is not a fixed number as there is a high degree of variance between women as well as between menstrual cycles. Normal menstrual cycle is typically presented as a range, which also has shown variance in the literature, 25 – 34 days (Mihm et al), 23 – 32 days (Cole et al), or 26 – 35 days (De Souza et al).[18,19,20] The follicular phase is the interval from the onset of menses up to and including the day of LH surge (ovulation). The 'normal' follicular phase range is reported as 10 – 23 days.[19] Luteal

phase is typically determined by the difference between the cycle length and the follicular phase length, reported to range from 7 – 19 days.[19] Values reflecting exercising females confirmed with urinary hormone testing, have been reported as being 15.1 ± 0.5 days for follicular phase length and 12.6 ± 0.3 days for luteal phase length.[18] Again these ranges are considered normal. This stresses the importance of using hormonal markers to assist with self-reporting methods to ensure the physiological function of menstruation is occurring and to adequately classify menstrual dysfunctions and disorders defined using phase lengths and biomarkers.[18]

The specific hormonal criteria to indicate the different menstrual cycle events are also provided in ranges. Table 1.0 provides the clinical serum ranges cited for estradiol and progesterone across the different phases of the menstrual cycle.[21]

Table 1.0 Clinical serum ranges for estradiol and progesterone

Estradiol	Conventional Units (pg/ml)	CF*	SI Units (pmol/L)	**Progesterone**	Conventional Units (pg/ml)	CF*	SI Units
Early Follicular	20 - 150	3.67	73 – 550	Follicular Phase	< 1.4	0.0318	< 0.0445
Later Follicular	40 – 350		147 – 1285				
Midcycle Peak	150 – 750		550.5 - 2753				
Luteal	30 – 450			Luteal Phase	3.3 - 26		0.105 – 0.827
				Midluteal Phase	4.4 - 28		0.140 – 0.890
Postmenopausal	<20		< 73	Postmenopausal	< 0.7		< 0.022

*CF: conversion factor

**NOTE: (A further discussion relating to hormone measurements during the menstrual cycle are presented in the review of literature under 'Methods used for assessing hormonal values across the Menstrual Cycle')

Quantifying GnRH is difficult, as it is not secreted directly into an easily measured fluid (i.e. urine, blood, saliva). LH acts as a surrogate marker for assessing menstrual function and the indirect measure of GnRH pulsatility. LH pulsatile pattern can be measured in the blood and urine. Changes in the regular LH pulsatility pattern has been the hallmark marker used to discern menstrual dysfunction in various female groups and the marker used to indicate that an energy availability threshold in females affects menstrual function in a nonlinear fashion.[7,11]

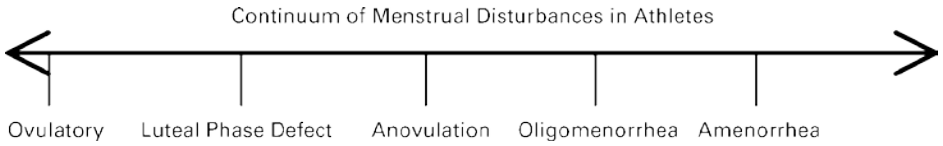
Table 1.1 provides a description of these pulsatile characteristics during the follicular and luteal phases.[22]

Table 1.1 LH pulsatile characteristics during the follicular and luteal phases

Phase	**LH Pulsatility**	
Follicular	↑ Frequency	↓ Amplitude
Luteal	↓ Frequency	↑ Amplitude

Menstrual Patterns: Exercise associated menstrual disturbances

For female athletes, menstrual patterns have been described on a continuum to illustrate the level of disturbance, ranging from ovulatory cycles to the most clinically severe being amenorrhea. A female athlete may fluctuate between ovulatory, luteal phase defects, and anovulation quite frequently.[23] However, a specific menstrual classification may not need a preceding pattern to occur to progress along the continuum. For example, oligomenorrhea does not need to occur before amenorrhea presents. Each menstrual state is defined in the following section.



Source: De Souza, MJ. Menstrual Disturbances in athletes: a focus on luteal phase defects. Medicine and Science in Sports and Exercise 2003; 35(9): 1556

a) Eumenorrheic / Normal / Regular Menstrual cycle

As stated above, there is not a consensus of an exact range or number of days that constitute a 'normal' menstrual cycle. Solely relying on self-reporting methods will likely include some forms of menstrual disorders.[18] Research that collected data over multiple cycles showed that even with cycles falling into the 'normal' range, hormonal confirmation provided evidence of menstrual dysfunction.[23] In addition, criticism of earlier research is that examining a single

menstrual cycle would likely underestimate cycle abnormalities or miss them entirely due to the known limitations of self-reports. As well, when single cycles are examined the intra-individual variability is not captured as it has been shown that the hormonal profile of a menstrual cycle can vary from one to the next.[23] To accommodate these findings eumenorrheic menstrual status is defined as self-reported cycles ranging in length between 25 – 35 days over 2-3 consecutive cycles with the inclusion of appropriate physiological biomarkers (i.e. hormone testing) to rule out menstrual dysfunctions.

b) Ovulation

Ovulation occurs when a mature egg is released from the ovary. This event happens midcycle and marks the beginning of the luteal phase. Specific hormonal criteria used for its detection is a LH surge concentration above 25mIU/ml and the estradiol peak concentration above 35ng/ml with a peak progesterone concentration above 5ug/ml during the luteal phase.[18]

c) Luteal Phase Defects

Luteal phase defects (LPD) are a classification of menstrual dysfunction defined as a reduction of the luteal phase length and/or a reduction in progesterone levels during this phase. LPD does not manifest through any visible outward symptoms for the female, i.e. cycle length, decrease in bleeding, and therefore needs to be confirmed through hormonal sampling. LPD is defined as short when the luteal phase length is <10 days or inadequate when the sum of the 3 day midluteal peak progesterone (PdG) <10 ug/ml and PdG peak concentration is below 5 ug / ml.[18, 23]

d) Oligomenorrhea

Oligomenorrhea is defined by irregular and inconsistent menstrual cycles lasting > 35 but < 90 days.[18] Recent studies investigating hormonal characteristics of oligomenorrhea in exercising females have identified hyperandrogenism as a possible alternative explanation for this condition as the hormonal profiles do not necessarily fit the hypothalamic inhibition and caloric deficiency seen in other disorders.[24,25,26] The previous notion that oligomenorrhea was a prelude to amenorrhea may not hold true in certain situations (reader is referred to the review by Awdishu et al, for more information.[27]

e) Anovulation

Anovulation refers to the absence of ovulation where the ovum is not released from the mature follicle. This usually occurs when a LH surge is not detected, in conjunction with low levels of progesterone in the luteal phase. De Souza et al, experimentally defines anovulation as "a cycle in which minimal increases in estradiol are observed concomitantly with a failure of LH to rise at midcycle, or when a luteal phase exhibits no increase in PdG concentration from a 5 day follicular phase baseline or when the peak PdG value is below 2.49 ug/ml".[18] As like other menstrual disturbances, monitoring with self-reports and/or menstrual bleeding may not capture anovulation.

f) Amenorrhea

Amenorrhea denotes the absence of menses for 3 consecutive months. The menstrual cycle and the hormonal milieu needed to support the feedback loop is absent i.e. failure of ovarian follicular development, ovulation, luteal function and no endometrial proliferation occur.[21] Primary amenorrhea, as defined by the American Society of Reproductive Medicine, is the absence of menstrual cycles in a girl who has not menstruated by the age of 15 years, but has

undergone other changes that reflect puberty.[28] Secondary amenorrhea refers to the cessation of menstrual cycles sometime after menarche. The definition most commonly used in the literature examining exercising females is failing to show signs of menses for at least 90 days (3 months).[18] In exercising females amenorrhea usually results from ovarian function being suppressed by LH pulsatility changes due to low energy availability. It is therefore generally referred to as functional hypothalamic amenorrhea.

Oral Contraceptive Pills (OCP)

OCP are a convenient and effective form of birth control for females. OCP prevent pregnancy primarily through suppressing ovulation by systemically controlling the endogenous amounts of estrogens and progestogens.[29,31] This is achieved with the levels of exogenous sex hormones provided from the OCP inhibit the pituitary secretion of gonadotrophins.[29,31] The progestin component in an OCP may also thicken cervical mucus, decreasing tubal motility and reducing the ease for sperm to pass. As well, progestins act to thin the endometrium making the tissue less receptive to implantation.[30] The estrogen component improves cycle control and also plays a role in preventing the development of the dominant follicle.[30]

The initial 'pill', first legalized in Canada in 1969, has since gone through numerous transformations and females are continually confronted with various formulations and products. For example, the 21 or 28-day formulas contain both hormone and hormone free pills or days. This brief withdrawal from exogenous hormones is enough to induce a menstrual cycle each month. Justification for these hormone free pills is questionable and some feel they were originally designed to obtain approval of the pill. For the female taking these formulas it offers the reassurance that she is not pregnant. In addition potentially calming concerns about the pills impact on reproductive function and long-term fertility.[32] The current market preparations of

combined OCP practically all contain the same estrogenic compound, ethinyl estradiol (EE) while some 24 different synthetic progestin compounds exist.[33]

Metabolism of contraceptive hormones: Ethinylestradiol (EE) and Progestins

The main estrogens produced by the body are estradiol, estrone, and estriol, the latter two forms are metabolites of estradiol that are produced in the liver. The reduced activity of estradiol given orally prompted the discovery of other forms of orally effective estrogens. An addition of an ethinyl group at the 17th position provided this option. Like endogenous estradiol, EE undergoes hepatic first pass metabolism and enterohepatic recirculation. Prior hepatic involvement, absorbed EE is rapidly conjugated to a glucuronide, which is inactive and undergoes renal excretion. The absorbed EE is also conjugated to sulfate compounds that are partially deconjugated to EE during hepatic recirculation. A mixture of both metabolized and unmetabolized compound reaches the liver.[33] In the liver EE undergoes oxidation and sulfuration. The most dominant oxidation reaction is 2-hydroxylation catalyzed by cytochrome P-450 enzymes. Unlike endogenous estradiol, the 16α-hydroxylation seems to be blocked by the ethinyl group. Other minor hydroxylated metabolites of EE have been detected but add little to the products of EE metabolism.[34] The amounts of EE entering the system after hepatic first pass ranges between 25 – 65%. Circulating endogenous estradiol is somewhat protected from metabolism because of binding to sex hormone binding globulin (SHBG), the other estradiol metabolites are bound to albumin. EE and EE sulfates are bound to albumin and none are bound to SHBG. (Refer to Appendix I for EE metabolism)

The progestins in combined OCP are classified by their chemical structure either being related to progesterone or testosterone. Figure 1.1. from Edelman et al, offers a chart of the progestin classification. The circled progestins are the types included in this study.[33] Progestins

not only act on the progesterone receptor but also interact with other steroid receptors (i.e. estrogen, androgen, glucocorticoid, mineralocorticoid) ultimately impacting and influencing other metabolic parameters.[35] For example, cyproterone acetate has been found to be a potent anti-androgenic progestin while drospirenone is anti-mineralocorticoid compound as well as having anti-androgenic properties.[35]

The progestins structurally related to testosterone can be grouped according to ethinylation at carbon 17. Drospirenone is the only non-ethinylated compound included in this study. The other testosterone related compounds fit the ethinylated groupings. Desogestrel, norgestimate and gestodene are derivatives of levonorgestrel.[33] A large variation in absorption and metabolism exist with these progestins. These factors affect the resulting bioavailability and thus one reason for the varying doses of each compound in the different pill formulas. Most are rapidly absorbed but not all undergo hepatic first pass effect (levonorgestrel and gesterone being in the latter group). Norethindrone, desogestrel, and norgestimate are all prodrugs and are converted to the active metabolites at varying degrees and rate.[33] The compounds structurally related to progesterone are less used for contraception purpose. Only medroxyprogesterone acetate and cyproterone acetate are used for this purpose.

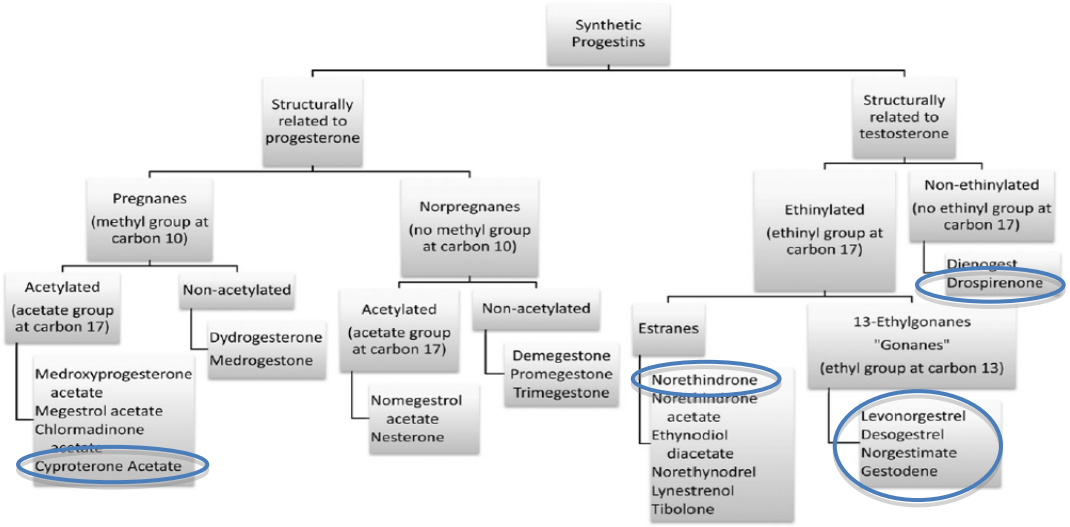


Figure 1.1 Classification of Progestins. Circled compounds are those included in current study

Pharmacokinetics of oral contraceptive hormones

Pharmacokinetics is defined as the movement of a drug through the body taking into account its absorption, distribution, metabolism, and excretion. Factors that influence one or all 4 of these processes include sex, age, dietary habits, alcohol intake, starvation, coadministered drugs, altitude, infection, disease, seasonal and circadian rhythms, pregnancy, and body weight.[33] It has been shown that the pharmacokinetics of OCP are highly variable. This variability is large between individuals; within an individual from day to day as well as across ethnic groupings.[34] Both the estrogen compounds and the differing progestins exhibit pharmacokinetic variability.

In the first hour after ingestion about 90% of oral EE is absorbed from the stomach and upper intestine. Peak blood level of EE is usually reached within 2 hours in some but has been observed to take as long as 6 hour in others.[33] This difference has been attributed to intersubject variability in 2-hydroxylation of EE, possibly due to the wide range of levels of the cytochrome P-450 enzymes that occur among individuals.[34] The bioavailability, defined as the amount of EE reaching systemic circulation after hepatic first pass metabolism, ranges between 25 – 65% of the amount ingested. The elimination half-life of EE is also variable, ranging between 6 to 27 hours.[34] Ethnic groups show variations in the pharmacokinetics of EE. Groups of women from Nigerian, Sri Lanka, Singapore, Thailand and the United States had differing plasma levels of EE, the differences remained even when corrected for body surface area. The assessment of the urine metabolites also varied across these groups, the lowest values were measured in Nigerian women and the highest in women from Thailand.[34]

Progestins also have high pharmacokinetic variability. They are well absorbed with an average maximum concentration reached within 1 – 3 hours.[33] The range of bioavailability of these progestins is large. The highest bioavailabilities are observed with levonorgestrel and gestodene, >90%, where the other progestins on the testosterone groupings are ~ 20% lower.[33]

Half-life times between the progestins range between ~ 8 – 80 hours, the lowest on the range being norethindrone and the highest that of cyproterone acetate (50-80 h) and drospirenone (~ 30 h) with the other progestins falling somewhere in between (12 – 24 h).[33]

In the current study the factors likely to contribute to varying pharmacokinetics between the participant groups are body composition, exercise, and dietary habits.

Androgenicity

Androgenicity refers to the ability of the progestin to produce masculine characteristics. An androgen is any natural or synthetic compound that through binding to androgen receptors stimulates or controls the development and maintenance of masculine related characteristics. These androgenic effects include acne, unwanted facial hair, oily skin and weight gain. Progestin androgenicity may also have an impact on lipid metabolism. Each progestin has a calculated androgenic activity related to the relative binding affinity to these receptors.[29] Androgenicity is calculated by multiplying the progestin dose within the OCP to the androgenic activity value ascribed to the specific progestin. For example, the OCP Alesse®, contains the progestin levonogestrel in an amount of 0.10 mg per pill. The androgenicity is then calculated as 0.10 mg x 8.3 = 0.83, where 8.3 is the assigned androgenic activity score. Based on previous research and cutoff values for high androgenicity, OCP included in this study needed to have a calculated value of ≤ 1.[29,36] There is some indication that androgenicity may have a more significant impact on performance and therefore may impact how a female athlete responds to training and possibility energy consumed.[29]

Table 1.2. Progestin properties at 1 milligram dose[36]

Progestin (1 mg)	Progestational Activity*	Androgenic Activity*
Norethindrone	1.0	1.0
Norethindrone acetate	1.2	0.6
Ethynodiol diacetate	1.4	1.6
Levonorgestrel	5.3	8.3
dl norgestrel	2.6	4.2
Norgestimate	1.3	1.9
Norelgestromin	1.3	1.9
Desogestrel	9.0	3.4
Drosperinone	1.5	0.0

* Progestational and androgenic activity are relative to 1-mg dose of norethindrone.

OCP come in a variety of types and formulations and therefore differ in the physiological impact. The reasons for a female to choose one formula over another depends on a number of factors including body composition changes, premenstrual symptoms, medical conditions, lifestyle, physician recommendations, and/or influence on athletic performance. Two recent papers reviewed the interplay between OCP use and performance in female athletes.[29, 31] Few studies have been conducted in this area so little is known about the impact of varying OCP regimes on performance. The authors of both studies stressed the importance of using similar types and formulation of OCP per study to prevent confounding variables in the results. This includes looking at whether the formulation is monophasic (same dose of hormones over the pill cycle) or multiphasic (varying hormone levels during the pill cycle), the concentrations of hormones (both estrogen and progestins) and thus its androgenicity and potency.[29, 31] That being said, the pharmacokinetics of OCP hormones reduces the researchers ability to make the above assumptions even with using similar formulas. Since much of pharmacokinetic variability is out of the researchers' control and capturing an accurate picture of the nutrition and activity regime without intervention was a key component to the research, an assortment of formulations were accepted. In addition, the type of progestin was not limited to one classification group or sub-grouping. Females in this study had been taking the same OCP formulation for at least 3

months. The timing of the intake and exercise records and baseline testing all commenced during specific days of the pill cycle across both consecutive months to reduce the factor of hormone variability. The OCP included in this study were: a) combined monophasic or triphasic brand b) 28 day pill cycles c) a low dose synthetic estrogen (ethinylestradiol) defined < 35 mcg and d) a calculated progestin androgenicity value ≤1.0 as per the calculation methods of Greer et al.[36] Higher androgenicity values may have the potential to alter performance and therefore this cut-off value will be used as suggested to reduce this possibility.[29]

Operational Definitions

Athlete

Currently there are not standardized parameters in research to define a female athlete sample group. Common markers consistently used either alone or in combination are: VO_2max values, VO_2 peak values, mileage/week, competitive level (i.e. competing at provincial or national level), body mass index, body composition measurements, and/or exercise energy expenditure.[24,25,26,37,38,39,40,41] The type of athlete that often presents with menstrual disturbances are aerobically trained athletes, runners, cyclists, and triathletes.[37,38,39,40,41] VO_2max values for athletes typically exceed those of sedentary females, especially when comparing against an aerobic-based sport.[42,43] Criticism of VO_2max as a marker is the large genetic or hereditary component, the poor predictability of certain types of performance, and the wide range of results from either a sport-specific testing method or one that does not reflect the mechanics or muscle use of the sport (i.e. treadmill test for swimmers).[42] Even so values for the athletic groups are higher than that of sedentary counterparts.[42,43] Wilmore, 1982, provides a review of studies assessing the aerobic capacity of females across varying sports.[42] The lowest values are reported for gymnasts (one value of 36.3 ml/kg/min), ballet (41.5 and 43.7 ml/kg/min), swimmers (40.5,

43.4, and 46.2 ml/kg/min) and tennis (44.2 ml/kg/min).[41] Most of the other sports were reported as having a VO_2max > 45 ml/kg body mass/min. The highest values were reported for cross-country skiing athletes (range from 57 – 68 ml/kg/min). It is therefore important when using a definition for a female athlete that VO_2max is not the only criteria considered. It is those sports with some of the lower recorded VO_2max values (dancers and gymnasts) that have been considered at higher risk for developing components of the female athlete triad[8] and a higher incidence of menstrual disorders i.e. females of lower body weights, younger ages, and aesthetic sports.[28] Including the additional component of training and/or mileage/week helps to include those sports that may not be aerobically-based. Therefore in defining the female athlete, 2 of the following 3 criteria were adopted for this study: a) has competed at a provincial, national, and/or international level, b) have a VO_2max > 50 ml/kg/min, and/or c) have structured training for greater than 10 hours/week.

Maximal Oxygen Consumption (i.e. VO_2max, maximal aerobic power, maximal oxygen uptake)

Maximal aerobic power is defined as the point at which oxygen uptake plateaus with an increasing workload.[45] Further increases in workload will not raise the oxygen consumption. Maximal aerobic power involves directly measuring oxygen consumption while the subject performs a graded exercise to a maximum. Different testing modes exist but the standard that typically yields the highest results is treadmill exercise.[48] Absolute values are expressed in litres of oxygen per minute (L/min). To provide a more comparative unit of measure across exercise testing modalities and individuals of varying body size, relative values are used; expressed as ml of oxygen consumed per kg of body weight per minute of exercise (ml/kg/min). [48]

Energy Intake (EI)

EI is the sum of all the measurable components in the food and liquid consumed during a specified time. The unit of measure for energy is typically expressed as calories or joules. The components in food measured and quantified as calories or joules are carbohydrate, fat, protein and alcohol.[44] One calorie or kilocalorie (kcal) is equivalent to 4.1868 joules

*Fat Mass, Fat Free Mass, Lean Body Mass**

a) Fat Mass is the absolute amount of fat that includes extractible adipose and other tissue.[46]

c) Lean Body Mass is defined as fat free mass including essential lipids.[46]

b) Fat Free Mass includes all residual lipid free chemicals and tissues including water, muscle, bone, connective tissue, and internal organs.[46] Some discrepancies exist in the current research surrounding EA measurement units using FFM or lean body mass (LBM). FFM is typically cited as the unit in the recent literature [49,50,51] but LBM is still presented as the relative unit by some researchers [6,52,53,54]. This study will only discuss EA in terms of FFM unit.

*Expressed in kilograms (kg).

Total Daily Energy Expenditure

Total daily energy expenditure (TDEE) is comprised of 3 components: a) resting energy expenditure, b) thermic effect of food and c) energy expenditure due to activity.

a) Basal energy expenditure (BEE) refers to the rate the body uses energy for all necessary processes to sustain life (i.e. respiration, heartbeat, renal function, and blood circulation) and the energy required to remain in an awake state .[44] It is expressed in kcal/24 hours. BEE is measured in a post absorptive state (fasted state for ~ 12 hours), supine position, and motionless state (shortly after waking in the morning). Resting energy expenditure (REE) is often a term used interchangeably with BEE but differs in collection methods and the subject's state. REE is

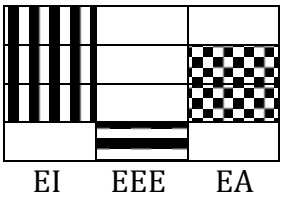
measured when the person is at rest in a comfortable environment, not necessarily upon waking. REE is typically 10% greater than BEE and accounts for about 65 – 85% of TDEE where BEE is thought to account for 50 – 70%.

b) Thermic effect of food or diet induced thermogenesis is the increase in energy expenditure above a resting value as a result of digestion, absorption and storage of food, typically accounting for 10% of TDEE.[45]

c) Energy expenditure due to activity includes intentional exercise bouts as well as non-intentional physical activity throughout the day. Exercise energy expenditure (EEE) is the value that makes up the energy used for deliberate engagement of a structured activity or training session(s) during a day and is the expenditure that was measured in this study. For the purpose of this study, REE and EEE were estimated and expressed in absolute (calories/day) and relative units of kcal/FFM kg/day.

Energy Availability (EA)

EA is defined as EI minus the EEE expressed per gram of fat free mass per day.[6,7] The diagram below provides a visual representation. If each box represents 1000 calories, EI would be 3000 calories, EEE would create a deficit of 1000 calories and what is left over would define the amount of EA = 2000 calories.



The literature has adopted EA in clinical practice, Table 1.3. provides EA calculations from a recent review on the female athlete triad.

Table 1.3. Examples of energy availability calculations

Example	Body weight (kg)	Body fat (%)	FFM (kg)	EI (kcal/day)	EEE (kcal/day)	EA = (EI-EEE)/FFM (kcal/kg FFM/day)
Low Energy Availability	61.5	13.5	53.2	1422	520	17
Weight Loss	61.5	13.5	53.2	2382	520	35
Weight Maintenance	61.5	13.5	53.2	2914	520	45
Carbohydrate Loading	61.5	13.5	53.2	3192	0	60

Abbreviations: FFM= fat-free mass; EI=energy intake; EEE=exercise energy expenditure

Source: Manore MM, Kam LC, Loucks AB. The female athlete triad: components, nutrition issues, and health consequences. Journal of Sports Sciences 2007; 25(S1): S612 – S71.

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CHAPTER 2
REVIEW OF LITERATURE

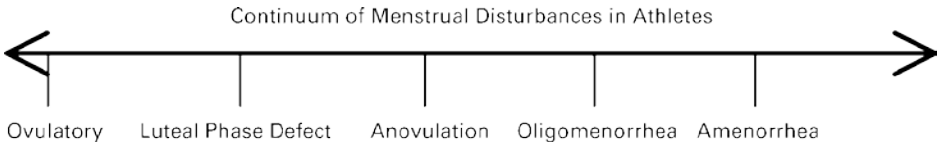
Introduction

Exercise-associated menstrual cycle disturbances are linked to an array of clinical consequences including decreased bone mineral density, increased incidence of stress fractures[1], reduced recovery from exhaustive exercise[2] and endothelial dysfunction[3]. The incidence and prevalence of these disturbances occur more in physically active females than their sedentary counterparts with higher rates appearing in aesthetic, endurance, weight class sports, younger aged athletes, higher training volumes and lower body weights.[4] Over the last 30 years research in this area made connections between these factors and menstrual function, EI, energy expenditure, body composition and the female athlete. To date, the EA theory provides the best evidence-based explanation. The series of well-controlled studies that demonstrated these reproductive disruptions occurred as a result of low EA and that exercise by itself, apart from the cost on EA has no disruptive effect were paramount in reducing support for the previous theories such as low body fat and exercise stress.[5, 6] However, the research indicating an EA threshold of 30 kcal/kg fat free mass (FFM)/day, below which reproductive markers were altered, was determined in a short-term laboratory setting, in healthy, sedentary females. Research has also emerged to suggest that the EA theory is not without limitations. Factors such as oral contraceptive use, gynecological age, chronic low calorie situations, and psychological stress in conjunction with or without energy stress have been found to affect how well the theory can be applied across individuals. In addition, to date this relationship has scarcely been examined in trained females. Recording dietary intake and exercise energy expenditure over long periods of time and invasive menstrual cycle monitoring procedures across more than one cycle has likely contributed to the paucity of research. Various methods exist to explore each of these variables and the

determination of the most suitable techniques to be used for athletes may encourage more researchers to pursue research in this area. In addition, tools that are feasible to use in training centres that include the assessment of menstrual status and EI will improve nutrition recommendations so that they are tailored more accurately for a female athlete.

Exercise-Associated Menstrual Disturbances

Menstrual dysfunction can be classified along a continuum. For female athletes the menstrual disturbances are generally identified as exercise-associated menstrual disturbances not associated with any anatomical or organic disease.[7] This distinction is necessary to ensure that the correct diagnosis of the menstrual dysfunction will lead to the appropriate treatment. The spectrum of exercise-associated menstrual disturbances is shown below.



Source: De Souza, MJ. Menstrual Disturbances in athletes: a focus on luteal phase defects. Medicine and Science in Sports and Exercise 2003; 35(9): 1556

New information exists that the spectrum does not necessarily act in a progression and that some forms of menstrual dysfunction (in particular oligomenorrhea) may exist as a phenotype of polycystic ovarian syndrome.[8] The most severe of these perturbations is amenorrhea, classified as functional hypothalamic amenorrhea where the absence of menses is due to the impairment of hypothalamic-pituitary-ovarian axis in generating necessary GnRH pulsatile secretions.[7]

Prevalence

The connection between menstrual function and structured physical activity only really surfaced when more females started to engage in physical activity. Early data came from surveys taken from the female athletes participating in the Olympics. In the 1964 Tokyo Olympics, 90% of the

female athletes reported regular menstruation compared to a dramatic rise of menstrual irregularities reported at 59% by female athletes for the 1976 Montreal Olympics.[9] Implementation of Title IX in the United States in 1972, mandating equal opportunities for both genders for any activity or education program receiving federal funding, also promoted opportunities for females to participate in sport. Researchers started to examine incidence rates in particular sports and found menstrual abnormalities (in particular secondary amenorrhea) existed in ballet, middle and long distance running, swimming, rowing, and field events.[9] Numerous researchers investigated the rate of menstrual irregularity but with little consistency in actual findings. A review on menstrual dysfunction and athletic women, Table 2.0, indicates that among those defined as athletes the incidence ranged from 0 – 50%.[10]

Table 2.0. Incidence of Menstrual Dysfunction in Athletes

Rougier and Linquette (1962)	Variable
Zhanel (1971)	12.8%
Kabisch (1972)	0%
Erdelyi (1962)	10 – 12%
Feicht et al (1978)	50% (marathon runners)
Dale et al. (1979)	34% (runners), 23% (joggers)
Speroff and Redwine (1980)	7.9%
Baker et al. (1981)	39%

Source: Baker ER. Menstrual dysfunction and hormonal status in athletic women: a review. Fertility and Sterility 1981; 36(6): 691 – 696.

A review by Loucks, summarized the incidence of secondary amenorrhea in the general population, runners and dancers.[11] Incidence rates in the general population were found to be consistency low (2 – 5%), consistently high in dancers (19 – 44%), and variable in runners (1 – 43%).[11] With the inclusion of more recent data the prevalence of menstrual disturbances in female athletes ranges from 1 – 64%.[14] This wide range has been attributed to the lack of standardized definitions, methods used to document menstrual status (being only self-report or a combination of self-report and daily blood or urine analysis), duration of monitoring (short term being 1 month to long term of up to or extending greater than 12 months), and selection bias.[13]

Very few studies have used hormonal measurements to assist in self-reports to correctly determine menstrual status. Confirming self-reported menstrual status with daily hormone measurements in a group of recreational runners, De Souza et al, showed a 3-month sample prevalence and incidence rate of abnormal menstrual cycles to be 48 and 79%, respectively.[14] More recently, De Souza et al, determined the prevalence of several menstrual disturbances using daily hormone measurements and self-report in a group of 67 exercising women (in a variety of sports, at both the recreational and competitive level) and 20 sedentary women.[12] They combined these data with a previously collected dataset that used the same criteria of measurements and definitions. They reported the prevalence of abnormal ovarian function (luteal phase defects and anovulation) to be 52% in the physically active women and only 5% among the sedentary women. This finding occurred despite the consistent inter-menstrual intervals of ~ 28 days. Secondary amenorrhea prevalence was 33.7% in the exercising women.[12] Previous studies relying on self-report have found the frequency of amenorrhea in exercising women to range from 1- 26%, indicating that self-reported data on menstrual status may not reflect an accurate prevalence among this population.[12] De Souza et al, used rigorous assessment methods for evaluating menstrual patterns in female populations by using consistent menstrual definitions, including luteal phase defects and anovulation in women across several consecutive cycles while verifying cycles with ovarian hormone measurements.[12]

Early findings from Loucks and since supported by De Souza et al, research examining the incidence rate of menstrual patterns in female athletes need clearly defined menstrual categories supported by hormonal verification that are then consistently applied to all populations being investigated (i.e. sedentary, general population and athletes).[11, 12] In summary, most studies have indicated the prevalence of menstrual disturbances in exercising females to be greater than that of

sedentary females with a higher incidence in aesthetic, endurance, and weight class sports, occurring at younger ages, higher training volumes and lower bodyweights.[4]

Physiological Consequences

Since the prevalence of menstrual disturbances in exercising women may be greater than 50%, it is important to understand the impact of these menstrual disturbances on health and performance. Prior to 1984 negative consequences associated with amenorrhea were not reported. Drinkwater et al, first showed findings that amenorrhea and the hypoestrogenic state were associated with low bone mineral density (BMD).[15] This study presented a comparison of regional bone mass at the lumbar vertebrae and at two sites on the radius between amenorrheic and eumenorrheic athletes matched for age, weight, height, sport and training regimens. They found significantly lower vertebral mineral density in the amenorrheic athletes but no difference at the radial sites. These findings started a flood of studies showing similar results. A recent review paper summarized that the majority of research shows a 10-25% lower BMD at the lumber spine in amenorrheic athletes compared to eumenorrheic controls.[1] The incidence of stress fractures is 2 – 4 times greater among amenorrheic athletes than eumenorrheic athletes. The premenopausal incidence of fractures of any type in non-athletes has recently been indicated as strong predictors of postmenopausal fractures, independent of BMD.[1] It is expected that females athletes have a 5 – 15% higher BMD than non-athletes.[1] Because of this, cut-off Z-score regarding BMD were recommended to be slightly higher for female athletes than postmenopausal women. The position stand for the Female Athlete Triad provides the following recommendations for female athletes: for 'low BMD for age' with risk factors a Z score between -1 and -2 and below -2 for osteoporosis in combination of risk factors for fracture.[1, 34] Low BMD in premenopausal women is not associated with the same fracture risk as low BMD for older women and the diagnosis of

osteoporosis is cautioned in premenopausal women solely on a BMD value. Therefore additional identifiable causes of bone loss in this population need to be considered.[1]

Bone health is just one area of negative clinical outcome due to the hypoestrogenic state of athletic amenorrhea. To date, investigators have explored many metabolic and hormone markers that are altered with increasing severity in menstrual disorders including decreases in resting metabolic rate, total T_3, leptin, insulin, IGF-1 / IGFBP-1, available glucose, and suppression of LH pulsatility, FSH, estradiol, and progesterone. Subsequently increases are shown in cortisol, ghrelin, and growth hormone.[16] How these perturbations relate to clinically known detrimental outcomes is still the focus of much of the research. Cardiovascular function, expressed as impaired endothelial function, has been reported as significantly reduced in amenorrheic athletes.[17, 18] Endothelial function is a marker used to predict cardiovascular disease (CVD) and future coronary events. These findings indicate that the hypoestrogenic state of athletic amenorrhea places the premenopausal females at risk for premature CVD.[18] Additional research has also provided promising information that this condition may be reversible to a normal state after nutritional intervention.[17, 18] Performance detriments of decreased recovery from exhaustive exercise, Harber et al, and just recently, reduced catecholamine response to high intensity exercise, Schaal et al. have also been observed in amenorrheic athletes .[2, 19] Implications surrounding less severe menstrual disturbances, i.e. luteal phase defects, may not affect bone health to the same extent but alterations to fertility, short or long term, have yet to be determined.[20] Long-term effects are often difficult to determine but research to date suggests that outcomes on bone health may be irreversible in previous amenorrheic athletes.[21]

Increased awareness in this area due to these negative implications has helped guide the focus of research to understand the mechanisms underlying menstrual dysfunction, identifying

consequences and progressing towards monitoring and intervention strategies for females.

Introducing the Energy Availability Hypothesis

The rising incidence of menstrual irregularities in female athletes led to the investigation of correlations between specific factors and those females reporting menstrual dysfunction. Some of these included duration and intensity of the activity, time of the athletic season, and weekly training mileage[9], prior menstrual dysfunction, nulliparity, stress, weight loss or alteration in body fat percentage, and age[10]. Feicht et al, showed a positive relationship between training mileage and incidence of amenorrhea, shown below, in a survey of 400 women on collegiate track and field and cross-country teams ($p<0.01$). Those running the lowest weekly mileage reported 6% amenorrhea while those running the highest weekly mileage reported 43% amenorrhea .[22]

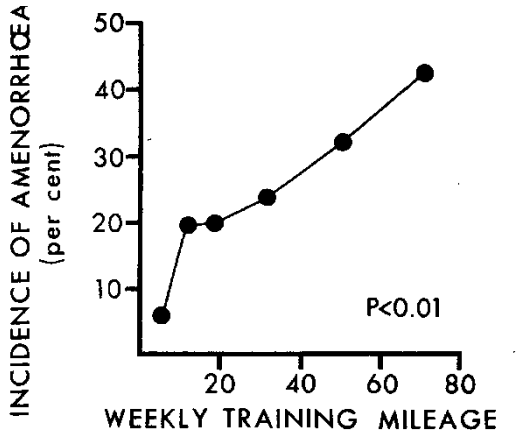


Figure 2.0. Correlation between training mileage and amenorrhea

Theories emerged around the underlying cause of these disturbances. One of the initial theories proposed that adipose tissue regulated reproduction function. From this theory recommendations that a minimum amount of fat was needed to initiate menarche (~17% fat/body

weight) and to maintain normal menstrual function (22% fat/body weight) were described.[23] A fat index was used to produce nomograms that could then predict critical weights for heights of females to maintain and restore menstrual function.[23] The evidence to support this theory stemmed from inferences made from research conducted in cattle and rats with limited evidence from human studies.[24] When studies compared eumenorrheic and amenorrheic athletes there was inconsistency among the research in terms of body weight and body fat comparisons between the groups. Some studies found significant differences while other research observed that both groups of athletes shared a common range of body composition.[11] Even the study conducted by Feicht et al (1978), the weights of the amenorrheic and regularly menstruating runners were not significantly different.[22]

Other theories suggested the stress of exercise as the 'culprit' in a female developing a menstrual dysfunction; that exercise may alter hormone concentrations and affect feedback mechanisms to the hypothalamus and pituitary. It was thought these alterations arose from one or all parameters of exercise i.e. distance, speed, frequency and/or duration.[11] Mechanistic evidence for this theory was lacking and it was not until 1998 when Loucks et al, demonstrated that exercise itself, apart from the energetic cost of the exercise bout, was not the reason menstrual function presented in female athletes.[6]

The metabolic fuel hypothesis initially was described in animals before adapted to a human model. Food availability becomes the primary cue (i.e. glucose and fatty acids) detected throughout the body by various metabolic sensors. These sensors provide feedback to the brain that directs the necessary signals to react to the fuel state of the organism. Low levels of fuel availability are known to disrupt ovulatory function and sexual behavior to varying degrees in human and animal models.[25] Warren et al, first explored the association of an 'energy drain'

concept in examining prepubertal dancers across 4 years. They found energy drain may have its impact on the hypothalamic pituitary set point and may act alone or in conjunction with a low body weight prolonging the prepubertal state and primary amenorrhea.[26]A few years later, Myerson et al, documented lower resting metabolic rate in amenorrheic athletes compared to their eumenorrheic counterparts and suggested that in an act to maintain a stable weight the body conserved energy even in light of a high caloric demand from their exercise without a compensatory increase in EI.[27]

This introduction of the 'energy drain' theory for female athletes in combination with the work completed on the metabolic fuel hypothesis has led to what is now identified as the 'Energy availability (EA) Hypothesis'. Where EA is defined as EI minus EEE. EA is the 'energy' or metabolic fuel left over for other functions outside of exercise, which include cellular maintenance, thermoregulation, locomotion, growth, and reproduction. In a series of well-designed studies, Loucks et al, examined the effect of EA on different metabolic hormones (i.e. cortisol, growth hormone, insulin like growth factor-I, thyroid hormones, insulin, FSH) and luteinizing hormone (LH; a key marker of reproductive function).[5, 6, 28] The conclusions provided the evidence that menstrual function (LH frequency and amplitude) was impaired with short-term low EA and exercise itself, apart from the cost of the exercise bout has no impact on menstrual function.[5, 6, 28]

These researchers also established an EA threshold, i.e. the menstrual status biomarkers elicit change at a specific EA value. Participants were healthy, young, regularly menstruating, habitually sedentary females. They completed a fixed and standardized amount of exercise over several days while EI was controlled to achieve the necessary EA for each group (Figure 2.1.).

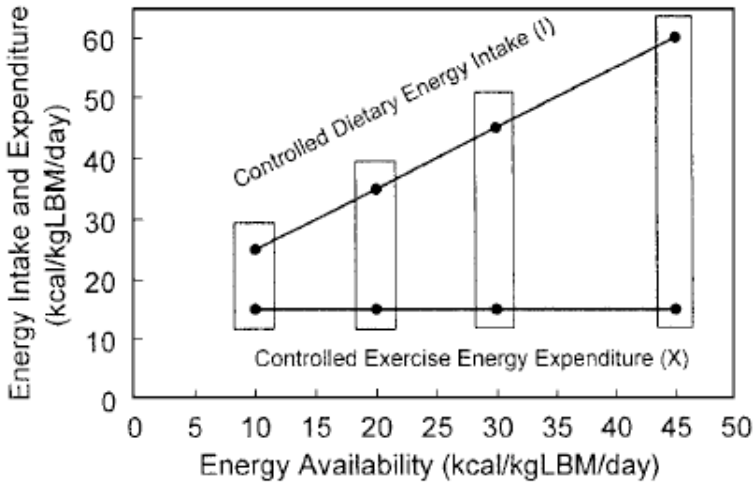


Figure 2.1. Experimental design of Loucks et al, 2003 [29]

All exercise sessions were supervised with EEE controlled at 15 kcal/kg FFM/day for each participant while EI was controlled to achieve EA of 10, 20, 30, 45 kcal/kg FFM/day.[29] The protocol was performed over 5 days and blood samples were obtained after the last day of exercise. All hormones investigated, including LH pulse characteristics, were disrupted at an EA less than 30 kcal/kg FFM/day and greater disturbances were detected in those subjects with the shortest luteal phases (i.e. 11 days).[29]

An EA threshold value has also been observed for bone turnover markers. Ihle and Loucks, used the same protocol as described above, a 5-day protocol in young sedentary healthy females, regularly menstruating females, assigned to varying EA groups.[30] Bone turnover markers were measured at the beginning and end of the 5 days. Bone formation markers included plasma osteocalcin (OC) and serum type 1 procollagen carboxyl-terminal propeptide (PICP) and the bone resorption marker was urinary N-telopeptide. The EA threshold for bone turnover markers was also around 30 kcal/kg FFM/day, however the response of the different markers was not uniform. The bone resorption marker increased greatly when the EA was 10 kcal/kg FFM/day

but not above the EA values 20 and 30 kcal/kg FFM/day. The bone formation markers were suppressed at all levels of energy restriction, with exception of PICP that showed a linear suppression across the EA groups and OC decline occurred between 20 – 30 kcal/kg FFM/day.[30]

EA threshold: Is this the only explanation for menstrual disturbances?

Previous research has been pivotal in demonstrating the association between EA and reproductive function in females and providing evidence that exercise alone has no suppressive effects on reproductive function apart from its energy cost.[29] The minimum EA threshold value of 30 kcal/kg FFM/day, as discussed earlier has been adopted as a recommendation for female athletes to support reproduction and bone health.[31,32,33,34] However, the research identifying an EA threshold was completed in a laboratory setting, using sedentary subjects for a short period of time. These findings have not been established in trained female athletes. Examining the EA threshold in an athletic population, in a typical training setting and the relationship to menstrual status confirmed using hormonal measurements is lacking.

Recent research has uncovered possible limitations of the EA threshold value under certain circumstances and participant groups. The possible connection of these new contributions will further lead to understanding modifiable aspects for exercising females.

Chronic Calorie Restriction

Chronic states of calorie restriction and the adaptations that may exist in trained females when exploring EA threshold is limited. The 5-day protocols using varying EA at a restrictive level may not translate to more chronic energy restriction states. Controlling for all the variables of EA over a long term would be difficult to conduct in humans, however studies in rhesus monkeys have provided some insight into these adaptations. In a cross-sectional study, Lane et al, examined the effect of chronic energy restriction of ~ 30% over 6 years on skeletal and

reproductive markers in premenopausal and perimenopausal rhesus monkeys.[35] The monkeys were divided into 2 groups, control (n=21) and energy restriction (ER) (n=19); the ER monkeys were provided 30% less food/day than the control monkeys for the 6 year time span. The ER monkeys still received adequate micronutrient intake and the reduction was in total EI not a deficiency of a specific nutrient. Housing and timing of meals were similar between groups. At the beginning of the study the monkeys were matched for age and body weight. Dual energy X-ray absorptiometry (DXA) scans were used to measure total body, lumbar spine and forearm bone mineral density. Early follicular blood samples were collected during a 3-month period (estradiol, FSH, progesterone, and LH). Serum samples were also drawn to measure ostecalcin, parathyroid hormone, 1, $25(OH)_2$-D and 25-hydroxyvitamin D. Urinary samples were collected to determine the bone resorption markers pyridinoline and deoxypyridinoline. The results showed that chronic ER for 6 years did not alter biochemical markers of skeletal metabolism, bone mass at any of the sites measured, and did not disrupt menstrual cycling or reproductive hormone levels. The ER monkeys did not differ significantly from control monkeys at any age.[35]

Williams et al, addressed the concept of calorie restriction and exercise, menstrual function, and gynecological age in females across ~6 months.[36] In a controlled exercise and diet intervention study, sedentary premenopausal women, 25 – 40 yrs of age, were divided into two groups, either a light conditioning group (LC) (2 supervised training sessions of 30 – 60 min/week and kept in a eucaloric state), gynecological age 22.4 +/- 1.1, or an exercise combined with caloric restriction group (EX +CR) (4 supervised training sessions 30 – 60 min/week plus a diet representing a 20 – 35% calorie restriction below baseline energy requirements), gynecological age 18.7 +/- 18.7. Subjects were assessed for 6 months to track the degree and time course of changes in menstrual status and ovarian steroid exposure levels (self-report,

ovulation detection kits and daily urine samples), and body composition (hydrostatic weighing). The results revealed a reduction in ovarian steroid exposure (15% and 20% reduction in serum estradiol and urinary E1G, respectively) but without alteration in the cyclicity, i.e. no significant changes in the average menstrual cycle length, follicular phase length, or luteal phase length were observed in either group. Significant increases in VO_2max, decreases in body weight (-3.7 +/- 0.5 kg) and percent body fat (-4.5+/- 0.7%) occurred (primarily attributed to changes in the EX + CR subjects).[36] The findings provide evidence that the reduction in ovarian hormones occur following an exercise and diet restriction intervention without a disruption in menstrual cyclicity.[36] EA value was not provided in this study and therefore interpretations of the 30% calorie restriction in reference to the EA threshold would be speculative. But it does provide additional evidence that women undergoing exercise and calorie restriction for a longer period of time (6 months) retain menstrual regularity. Also of importance in this study is the gynecological age of the subjects. Gynecological age has also been explored as a possible independent aspect affecting the response of EA on reproductive function.

Gynecological Age

Loucks et al, found differences between the relationship of LH pulsatility and EA in groups distinguished by gynecological age.[37] LH pulsatility was not affected by a 10 kcal/kg FFM/day in the group with gynecological age > 14 yrs but was with a gynecological age between 4 – 8 yr. Earlier studies documenting an EA threshold at 30 kcal/kg FFM/day were reported using healthy sedentary individuals with a gynecological age closer to 8 yr limiting the external validity of the EA threshold of 30 kcal/kg FFM/day to females who are sedentary with a younger gynecological age.[29] Considering these above findings, a normal LH pulsatility could exist between 10 – 30 kcal/kg FFM/day depending on a female's gynecological age and training status. In fact most

studies evaluating EI and menstrual function used females with younger gynecological ages less than 14 years (Ihle et al; 8.6 yrs, Barrack et al; 1.9 – 2.9 yrs, Loucks et al, 2003; 8-9 yrs, Loucks et al, 1994; 7.5 yrs, Loucks et al, 1998; 8.7 yrs).[30,38,28,29,6] It appears in some studies the eumenorrheic athletes compared to the amenorrheic athletes fit the gynecological age profile of the above study, Laughlin et al, menstruating athletes gynecological age = 17.3 ± 1.4 years and amenorrheic athletes 11.9 ± 1.2 years, De Souza et al, gynecological age menstruating athletes 15 ± 1.0 years and amenorrheic athletes 9.3 ± 0.9 years).[12,39] The association with gynecological age and a possible decrease sensitivity to lower EA is not new; Rogol et al, found 17 women, gynecological age = 17.8 +/- yr., who increased running mileage up to 70 miles/week unaccompanied by an increase in dietary intake had no disruption in LH pulsatility and ovarian function.[40] It is not suggesting that low EA and subsequence menstrual dysfunction for athletes can not exist at higher gynecological ages, but it does suggest possible limitations to the EA threshold.

Psychosocial Stress and Synergistic effect of both types of Stressors

The initial 'stress theory' defined exercise as a stress or as a metabolic stress contributing to functional hypothalamic amenorrhea (FHA). Further research into the stress theory redefined stress as psychological in nature not exercise to explain FHA.[41] Elevated cortisol found in those with FHA suggested that the hypothalamic pituitary adrenal (HPA) axis activity was increased. This increase alone in cortisol or the increase in the HPA axis may not be the sole influence on ovarian function but may act to induce central changes that for those that also have a heightened responsiveness or sensitivity to stress may predispose them to FHA.[42] Therefore with FHA management, psychological therapies must also be included addressing psychological, psychiatric and behavioral variables.[42] The synergistic combination of psychosocial and

metabolic stressors on reproductive function was examined in cynomologus monkeys.[43] The study examined monkeys for 4 menstrual cycles, 2 cycles prior and 2 during the intervention; monkeys were randomly assigned to one of the following experimental groups; 1) group moved to an unfamiliar cage surrounded by unfamiliar monkeys (previously shown to induce a stress response) 2) group that exercised for 1 hr/day, 5 days/wk, at a moderate intensity also undergoing a 20% calorie restriction or 3) group undergoing a move (as in group 1) and exercise and calorie restriction (as in group 2). Prior to the study all monkeys were documented as having 3 consecutive regular menstrual cycles.[43] The results showed that the stressors (either mild psychosocial, mild diet restriction, and moderate exercise) disrupted menstrual function in a small percentage of the population (~10%) in intervention groups 1 and 2, but when combined as in group 3, 70% experienced menstrual dysfunction.[43] In a follow up to this study the researchers studied the use of a combination of approaches to addressed both psychosocial and metabolic stressors on FHA such as cognitive behavior therapy, stress management, relaxation techniques, adequate calorie intake. The success of these combined treatment approaches shows promise in treating women with FHA.[44] However, within the study design it is difficult to determine if it was indeed a combination of the treatments or if only one treatment establish regular menstruation.[44] To date, cognitive behavioral treatment has not been tested or used in the athletic realm. Pauli et al, highlight the importance of recognizing the impact of both psychogenic and metabolic stress on reproductive function and addressing both areas as needed to ensure long term restoration of menstrual function.[45]

Females Athletes and Oral Contraceptive Pills (OCP)

Research exploring EA in female athletes most often exclude females taking OCP. The pattern of hormones across an OCP cycle to not match a normal cycle nor do they fit among the other

menstrual function definitions. Only one study to date included a group of female athletes using OCP where EI and expenditure were determined.[46] With a growing number of athletes taking OCP, it becomes important to examine this group. In the late 1980s a study reported only 5 – 12% of athletes using oral contraception (OC).[47] Following this, Brynhildsen et al, found OC use in female team sport athletes to be 47%.[48] Other research groups indicated that the use of OC in the athletic setting matched that of the general population.[48] A national survey conducted in 2006 on contraceptive use of Canadian women found OC use to be 67% in those aged 15 – 19, 58% in the 20 – 29 age group and 32% in the 30 – 39 age group.[90] More recently, a survey conducted on 68 athletes from 15 different sports, found OC use at 83% in the elite athletes (mean age 25 yrs.).[49] Considering the above prevalence, likely more than half of athletes are using OC, it seems imperative that study designs incorporate subjects taking OC. The survey of the 68 elite Australian athletes, found their reasons for taking OCP were contraception (75%), cycle regularity (43%), control of menstrual symptoms (34%), cycle manipulation (32%), and other (4%).[82] Amenorrheic athletes may be recommended to use OCP as a hormonal replacement therapy to protect bone health, but such prescription remains controversial.[82] Since no studies have examined the EA of female athletes on OCP it begs the question as to whether the EA theory and threshold hypothesis holds true for this group of females. The study conducted by Thong et al, had four groups: recreational cyclic athletes (RCA), recreational athletes on OC (ROC), elite cyclic athletes (ECA), and elite amenorrheic athletes.[46] They reported the ROC, RCA, and ECA groups had similar EI where the ECA had significantly greater EEE and therefore a lower EA (~30 kcal/kg FFM/day) than both recreational athlete groups (~33 kcal/kg FFM/day).[46] Because this study's purpose was not to explore EA in female athletes on OCP, some of the factors to help distinguish differences were not present. There remains a large gap in

data exploring EA in female athletes using OC methods.

Thus far, the research indicates various factors to consider around EA: menstrual status needs to be determined using some form of hormonal confirmation, gynecological age, a psychogenic component may act independently or synergistically on the GnRH pulse generator and needs attention, chronic adaptation to a moderate calorie restriction may occur without implications to reproductive function, and the paucity of research including OCP use.

Determining a method that is less invasive and accurate and can be employed in an exercising setting may help to shed some light on modifiable factors for the individual. It becomes extremely important to use appropriate tools to measure EA to help determine the intervention that is most appropriate.

EA and Menstrual Status using noninvasive tools

Measuring EA and menstrual status in female athletes is challenging for researchers and burdensome for participants. Monitoring dietary intake is time-consuming and the results are subject to reporting error. Direct estimates of energy expenditure often require lab-based equipment. Menstrual status determination requires frequent and lengthy analysis of blood or urine. Previous studies have not fully explored this relationship of reproductive function and energy intake in female athletes. As a result they have not included EA (or its components), have relied on EI measures alone, and/or used self-report to determine menstrual status instead of daily measurement of reproductive hormones.[50,51,52,53,54,55,56,57,58,59,60,83]

These studies then compare the results to nutritional recommendations without consideration of energy balance and menstrual status typically concluding that the energy intakes for female athletes are sub-optimal and the majority of athletes do not meet the current sport nutrition recommendations. The most recent joint position stand on Nutrition and Performance developed

through the collaboration of Dietitians of Canada, American Dietetic Association, American College of Sport Medicine, provide general guidance for athletes and recommend specific carbohydrate amounts for athletes ranging between 6 – 10 kg/kg body weight/day.[31] Other reference ranges used for carbohydrate are 5-7 g/kg/day for general training days and 7 – 10 g/kg/day for periods of increased training and competition.[61,62] The newly published International Olympic Committee (IOC) consensus papers included one specifically addressing carbohydrate recommendations for training and competition.[84] This paper outlines 'situation-specific' carbohydrate needs, identifying amounts responding to low intensity or skill-based sessions to extreme durations of exercise of 4-5 h/day. The carbohydrate amounts for the light or low-intensity sessions are suggested to be 3-5 g/kg/day extending to the highest output category with amounts of 8 – 12 g /kg/day.[84] The most current recommended amounts do not differentiate between the male and female athlete. This position stand provides an EA recommendations for female athletes stating, "Many researchers have suggested that 30kcal/kg/FFM/day might be the lower threshold of energy availability for females".[31] Other sources provide more detailed EA values. Burke et al, states that normal healthy adults typically achieve an energy balance when EA is ~45 kcal/kg FFM/day and provides examples of how to use this value in reference to an athlete.[32] The current ACSM Position Stand on the female athlete triad mentions both the 30 and 45 kcal/kg FFM/day values but with no additional guidelines.[34] The review paper on the Female Athlete Triad by Manore et al, provides the most detailed EA recommendations for female athletes and suggest that "athletes should aim to maintain EA between 30 – 45 kcal/kg FFM/day for weight loss, near 45 kcal/kg FFM/day for weight maintenance, and >45 kcal/kg FFM/day for growth and carbohydrate loading".[33] Among the 2011 IOC consensus papers, one specifically on energy availability continued to support these ranges and added that "athletes should follow diet

and exercise regimes that provide energy availabilities of 30 – 45 kcal/kg FFM/day while training to reduce body size or fatness".[85]

Are these recommendations representative of the actual energy intake and EA of female athletes? A review paper by Burke et al, examined studies over a 30-year span (~1970 – 1990s) that reported the dietary data on elite athletes.[61] Carbohydrate intakes for female athletes were reported lower than men and the mean value was 5.5 and 4.7 g/kg/day for female endurance and non-endurance athletes respectively.[61] The authors concluded that female athletes struggle to meet the carbohydrate guidelines more so than males and fall short of achieving the optimal values recommended. The dietary intake data collected from female athletes over the last 10 years yield similar findings when comparing actual EI to sport nutrition recommendations. Lun et al, reported the calorie and carbohydrate intakes of elite Canadian female athletes to be sub-optimal in reference to the recommendations after analyzing 3 day food records of 201 female subjects mean age of 21.5 ± 15.8 years. The mean carbohydrate intake was 5.1 ± 1.8 g/kg body weight and calorie intake/day was 2303.6 ± 712.6.[56] Athletes were categorized based on type of sport (i.e. power, intermittent or judged) but the intake values were not expressed separately for males and females. Heaney et al, used a food frequency questionnaire in elite Australian athletes and found female athlete carbohydrate amounts lower (average of 4.5 g/kg/day) than the recommendations.[63] Much of the research exploring carbohydrate intake in female athletes has been conducted in those training in endurance sports. One study looked at the effect of variable carbohydrate intake on performance in female cyclists during an exercise trial using a double blind cross over study design with varying carbohydrate amounts of 3, 5, 8 g of CHO/kg body weight.[64] Eleven, 20-45 year old eumenorrheic female cyclists were randomly assigned to a eucaloric diet providing one of the three carbohydrate amounts. The compliance rate for food

intake was 92% and the researchers found most subjects were unable to adequately consume enough CHO to meet the 8g/kg/day requirements. No performance marker was statistically different between any of the three carbohydrate amount groups.[64] The collection of 3-day food records prior to the test protocol reported the mean intake of CHO to be 5.5. g/kg/day. Although this study does not compare a regular training period with a higher intense or competition time it questions the appropriateness of the current carbohydrate intakes for female athletes when trying to consider menstrual status, age, training level of the subjects, and exercise intensity.

Without coinciding the menstrual status of a female athlete to the dietary records, assumptions of energy needs not being met cannot be made. Normal menstrual status functions as a marker that the body is meeting its energy demands. The dietary intake value itself does not define the energy status of the athlete. The summary of the above studies suggests that few female athletes meet the recommendations described in the sport nutrition literature. But whether the athletes in the study are meeting their physiological needs cannot be answered.

Studies have also compared the EI between eumenorrheic and amenorrheic athletes. Table 2.1 presented by Myerson et al, shows only 1 study where the amenorrheic athletes had significantly lower intakes than the eumenorrheic group. The other 3 studies presented showed no difference.[27] Laughlin et al, confirmed menstrual status with hormonal measurements and found the food intake higher in the amenorrheic group than the sedentary control and the cyclic athletes.[39] It is difficult to compare these studies as the study design and definitions are not consistent and misreporting of food recording not always indicated. Further yet, there has been less research conducted where all the necessary variables are determined simultaneously to discern an energy balance or EA value.

Table 2.1. Summary of dietary intake of amenorrheic and eumenorrheic athletes[39]

Study	Amenorrheic		Eumenorrheic		kJ Difference*	Significance
	kJ	kJ·kg^{-1}	kJ	kJ·kg^{-1}		
Nelson et al. (15)	7242	126	9419	170	2176	A < E
Marcus et al. (14)	5325	107	7179	134	1854	NS
Drinkwater et al. (7)	6790	125	8225	143	1436	NS ($P < 0.06$)
This study	7242	141	8096	159	854	NS

* Additional daily kJ consumed by eumenorrheic (or cyclic) athletes relative to amenorrheic athletes.

Table 2.2 from Manore et al, provides studies where an EA comparison was made.[33] It is important to note that the authors evaluated the original research to calculate some of the values for EA in this chart. The calculations do not show statistically significant differences so the interpretation of these data should be approached with caution. The values show that all but one EA calculated value for all the groups (eumenorrheic and amenorrheic athletes) is below 45 kcal/kg FFM/day. The EA range for the eumenorrheic groups is between 19 – 33 kcal/kg FFM/day and the EA range for the amenorrheic group is between 16 – 28 kcal/kg FFM/day. There is only one study that explores an EA in females athletes taking OCP.[46] Thong et al, 2000, had four groups of athletes, one group consisted of recreationally active women taking OCP. Interestingly, the mean VO_2max for this group was 52.7 ± 2.1 with an average exercise expenditure of 579.9 ± 59.6 kcal/day. In other studies, these women would meet the athlete criteria. The EA calculated for this group was 33 kcal/kg FFM/day. More recently Scheid et al, determined EA in a group of healthy sedentary female subjects at baseline.

Table 2.2. Summary table of studies examining energy intake (EI), total energy expenditure (TEE), energy balance (EB), exercise energy expenditure (EEE), energy availability (EA)[33] Red outlined column highlights EA

Table II. Mean energy intake (EI), total energy expenditure (TEE), energy balance (EB), exercise energy expenditure (EEE), energy availability (EA), and macronutrient intakes in adult female athletes with and without menstrual dysfunction[a].

Reference	Menstrual Status (n size)	Athletes	Body fat (%)	BMI ($kg \cdot m^{-2}$)	FFM (kg)	EI ($kcal \cdot day^{-1}$)	TEE ($kcal \cdot day^{-1}$)	EB ($kcal \cdot day^{-1}$)	EEE ($kcal \cdot day^{-1}$)	EA ($kcal \cdot day^{-1}$)	EA ($kcal \cdot kg\ FFM^{-1} \cdot day^{-1}$)	EI ($kcal \cdot kg\ BW^{-1} \cdot day^{-1}$)	Protein intake ($g \cdot kg^{-1} \cdot day^{-1}$)	CHO intake ($g \cdot kg^{-1} \cdot day^{-1}$)	Fat intake ($g \cdot kg^{-1} \cdot day^{-1}$)
Tomten and Hostmark (2006)[a]	Irregular cycles (not classified as amenorrhoeic) ($n=10$)	runners	19.8	20.7	44.8	2318	2677	−359	526	1792	40	39.2	1.49	5.7	1.04
	Eumenorrhoeic (n=10)		18.1	20.0	41.1	2940	2629	+311	502	2438	59	51.9	1.71	7.0	1.70
Thong *et al.* (2000)	Amenorrhoeic ($n=5$)	runners & cyclists	14.6	18.9	44.8	1672	N.A.	N.A.	970	722	16	31.8	1.0	5.5	0.73
	Eumenorrhoeic ($n=8$)		15.2	19.3	44.8	2277	N.A.	N.A.	956	1321	30	43.0	1.5	6.8	1.20
Kopp-Woodroffe *et al.* (1999)[b]	Amenorrhoeic ($n=4$)	runners & cyclists	17.7	21.0	50.4	1892	2773	−881	645	1247	25	30.9	1.23	5.5	0.67
De Souza *et al.* (1998)	Eumenorrhoeic Ovulatoary ($n=24$)	runners	N.A.	N.A.	N.A.	1837	2075	−238	N.A.	1358	30	31.6	1.16	4.9	0.93
	Eumenorrhoeic LPD ($n=21$)			N.A.	N.A.	1993	2031	−38	N.A.	1522	32	35.0	1.23	5.1	1.12
	Eumenorrhoeic Anovulatory ($n=8$)			N.A.	N.A.	1326	2405	−1079	N.A.	1167	25	20.4	0.88	3.5	0.55
Laughlin and Yen (1996)	Amenorrhoeic ($n=8$)	runners & triathletes	16.0	19.4	46.4	2106	N.A.	N.A.	1074	1032	23	38.1	1.21	6.9	0.58
	Eumenorrhoeic ($n=8$)		15.9	19.6	48.9	1739	N.A.	N.A.	906	833	19	29.9	0.98	4.4	0.86
Wilmore *et al.* (1992)	Amenorrhoeic ($n=8$)	runners	10.8	18.4	45.8	1781	2433	−651	476	1305	28	34.6	N.A.	N.A.	N.A.
	Eumenorrhoeic ($n=5$)		10.3	18.7	46.6	1690	2305	−615	402	1288	28	32.5			
Myerson *et al.* (1991)	Amenorrhoeic ($n=7$)	runners	14.6	19.4	43.8	1730	1892	−148	526	1204	28	34	1.03	4.6	1.03
	Eumenorrheic ($n=10$)		15.0	19.5	42.9	1934	2128	−193	537	1397	33	38	1.34	5.3	1.31

[a]All participants had menstrual function confirmed by blood hormones; Tomten and Hostmark (2006) did not specifically screen for amenorrhoea. To convert $kcal \cdot day^{-1}$ to $kJ \cdot day^{-1}$, multiply by 4.184. All studies used 7-day food records except for Tomten and Hostmark (2006), who used 4-day records. All studies screened for eating disorders.

[b]Data averaged over four case studies at baseline.

BMI = body mass index ($kg \cdot m^{-2}$); EB ($kcal \cdot day^{-1}$) = [EI ($kcal \cdot day^{-1}$) − TEE ($kcal \cdot day^{-1}$)]; EA = EI − EEE; LPD = luteal phase deficiency; FFM = fat-free mass; N.A. = not available

Three different groups had EA values of 42.0 ± 7.4 SD for n=7, 43.9 ± 3.8 SD for n=5 and 45.1 ± 4.9 SD for n=10 (difference between these values were non-significant p = 0.574).[86] A cross sectional study by Reed et al, looked at the effect of increased caloric intake on menstrual function and bone health in regularly exercising women while also measuring EA of their subjects.[87] They categorized the subjects based on menstrual status, an ovulatory control group with n=13 and the other group having exercise-associated menstrual cycle disturbances (EAMD) with n=12. The calculated EA for a 3 day period was 42.1 ± 9.2 SD and 28.8 ± 11.5 SD, respectively (p = 0.006).[87] Two additional separate research papers measured the EA value of dancers.[88,89] Hoch et al, estimated the EA of 22 professional dancers across a 3 day period including 2 weekdays and 1 weekend day. The data were expressed in absolute terms and only the dancers that had a negative or low EA (described as negative) were reported. Seventeen dancers (77%) of the sample had low or negative EA (-547.8 ± 359.9 SD kcal/day).[88] Doyle-Lucas et al, also studied the EA component in dancers but compared the values to pair-matched controls. These values were 40.9 kcal/kg FFM/day for the eumenorrheic controls, 5.8 kcal /kg FFM/day eumenorrheic dancers and 0.6 kcal/ kg FFM/day dysfunctional dancers defined as having irregular menstrual cycles and /or amenorrhea.[89]

In summary, a measured value for EA in female athletes is sparse and few provide detailed menstrual classification. The current literature reports that healthy eumenorrheic sedentary females have greater EA than eumenorrheic athletes who have greater EA than female athletes with menstrual dysfunction. The reported EA values for both groups of athletes seem to be far less than for the weight maintenance EA outlined in the current recommendations. The amenorrheic athletes EA values estimated are all below 30 kcal/kg FFM/day and most of the eumenorrheic athletes EA values also fall below the threshold. Considering the high prevalence

of use of OCP in athletics the need to study this group is apparent. It appears from the available research that female athletes consume less carbohydrate and EI than the recommendations as well as a lower value of EA than that proposed in a sedentary, untrained research group. The use of reliable and less arduous measures of EI, energy expenditure and menstrual status would increase subject compliance and likely increase the data necessary to address the EA and menstrual status question in female athletes. These findings would then further our understanding of EA recommendations for female athletes. Gold standard methods may be present for the components of EA however; issues around practicality, cost, and athlete burden are often non-transferable from a laboratory setting. A review of techniques and methods commonly used in determining EA were explored to determine the most appropriate alternatives suitable for this study.

Energy Intake (EI)

EI is required to calculate EA. EI is determined using food intake assessment methods. The classic methods used to analyze food intake can be divided into 3 categories: 1) individual recall of food intake (e.g. 24 hour recall), 2) interview methods obtaining diet histories or retrospective questionnaires (e.g. food frequency questionnaires (FFQ), 3) dietary intake record (e.g. 1 – 7 day prospective food record collection).[66] Each method has its own instrument disadvantages and advantages but the most important consideration is which method represents the type of information needed for the study design. This study requires a method able to capture the intake of each individual with accuracy during specific periods of time. Retrospective questionnaires provide estimates on habitual dietary intake patterns at the group level and are most suitable for large scale epidemiological studies. It fails to determine specific caloric estimates for each individual which is needed to calculate EA. Food recall methods rely heavily on the memory of subjects and often fail to provide precise individual assessment for a period of time.[66] Koehler et

al, has been the only study to date that has assessed the validation of a combined assessment of nutrition and exercise protocol on an athletic group.[76] They used the Cologne nutrition and activity protocol where the food recording includes an adjustable list of foods and food specific serving sizes where the athlete marks the frequency of the particular food item throughout the recording period. The food intake component showed limited validity and large individual variation when compared to doubly labeled water and 24 h urea excretions.[76] They suggested this tool being more appropriate for group evaluations than with individual athletes as it did not provide the precision to assess on an individual level.[76] Over the last 60 years prospective food records have been typically used to assess EI in athletes and was selected as the method for the current study.[66] The justification for the use of this method was based on the recording period and participants. Misreporting issues were addressed by following the guidance and recommendations of previous research in this area.

Obtaining a valid food record is key in providing a close representation of the participant's habitual intake. A valid record includes one that is accurate and complete; including all food and drink consumed on the specified days where the choice of consumption has not been influenced by the act of recording.[67] Misreporting is one of the main sources of error in dietary assessment, consisting of both over and underreporting (either due to undereating and/or underreporting). Underreporting presents when a discrepancy between EI and energy expenditure occurs without a change in body mass; undereating is distinguished by eating less than usual or less to maintain body weight and results in a decline in body mass.[68] Factors that increase the likelihood of underreporting are subjects with a higher BMI, female, lower socioeconomic class, lower level of education, smokers, dieters and those with high food intake. Psychological factors, including perception of body image, how one is concerned with the

opinions of others, pressure to provide answers that are socially acceptable, and level of depression, have also been shown to increase the chance of underreporting. These factors are usually assessed in the form of questionnaires.[68] A study examining the characteristics of women who underreport found they were likely to score higher for social desirability and body dissatisfaction.[69] The athlete population shares similar factors as mentioned above.[65,66] Other research suggests differences in reporting between different sporting groups. Braakhuis et al, found greater day-to-day variability in food intake in weight-conscious sports compared to those athletes involved in endurance, team and power or skill sports.[70] Therefore, when determining EI in female athletes these factors need to be considered to minimize misreporting and provide support when possible to ensure the most accurate EI is collected.[65,66,70,71]

To identify misreporting usually the EI and EE are compared then the difference between the two measurements is calculated to determine the magnitude of misreporting. Therefore, the validation of an accurate food record rests on the assumption that EI must equal EE when weight is stable.[67] The magnitude of misreporting is usually expressed as a percentage, where a positive value indicates underreporting and a negative value is indicative of overreporting (i.e. EI – EE = % of misreporting). The various methods used to determine energy expenditure include doubly labeled water (DLW), urinary markers, cut-off equations, and comparisons with estimates or measured EE.[68]

DLW technique has been considered the gold standard to assess EE in subjects during free-living conditions however it is costly and requires sophisticated laboratory equipment. The Goldberg cutoff technique was developed to evaluate EI against presumed energy requirements where EI is expressed as a multiple of the mean BMR estimated from equations. It is best used at distinguishing bias at the group level and not the individual level, since the confidence limits

become much wider for small sample sizes (i.e. for an individual case n=1). For this study using the Goldberg cutoff values for n=1 and 7 days the 95% confidence limit values are 1.05 – 2.28, range of 1.23. This compared to a sample of n= 10 and 7 days where the 95% CL range is 0.38.[71,91] Thus at an individual level the sensitivity is poor and only extreme degrees of misreporting will be identified.[91] If EEE is calculated a researcher has a closer measured value compared to the physical activity level (PAL) that are assigned to the Goldberg cutoff and could compare EI directly to energy expenditure as a ratio of EI: REE+EEE.[71] However this value does not account for other daily activities and likely most of the sedentary group would have overestimated ratios with some days having little or no recorded purposeful exercise bouts. Monitoring body mass at the beginning and end of a food recording period, undereating could be detected at the individual level. Validation of EI by subjects is recommended due to the issues of substantial bias to under estimate food intake. Without these above methods EI can be expressed as EI: REE for comparison assuming that the weight of the individual has remained constant.

Other criteria have been suggested to improve the accuracy of the information obtained from food records. A 3-7 day food record for athletic individual assessment is generally recommended.[66] It has been shown that by using 7 day food records reliability of the measurements are improved; the average coefficient of variation for daily energy intake is ~23%, by choosing 7-days this value reduces to ~8.5% with 95% confidence limits of ± 17%.[90] In addition, Braakhuis et al, found a 2-3 fold reduction in the variability of nutrient intake for elite athletes when the recording period was increased from 24 to 3 days, and from 3 to 7 days.[70]

This study also addressed the error contributed by the nutrition coder. The nutrition coder, who inputs the food items, has an independent error above the variability of the subjects reporting. Using multiple coders, even well-trained and sport nutrition professionals need to

have standard protocols set out to maintain consistency within a study.[70] By factoring coder error into the estimation for sample size, it may reduce the sample size required to obtain significant results.[70] The following recommendations for collecting and assessing food records for the athletic population include: a) cross check subjects food records when possible to any misunderstandings or identify mistakes upon recording, b) take time for providing clear instructions to the participants on food recording c) familiarize oneself with the common foods and supplements used by subjects and obtain specific label information for brands d) create a standardized protocol for handling the coding of each entry on the food record, for quantifying and matching to items in the database and use protocol on future occasions e) develop a protocol for quality control of the processing of each food record, i.e. complete routine spot-check of each day's record for possible entry errors or values that may not seem appropriate.[70]

Exercise Energy Expenditure (EEE)

The other component necessary to determine EA is EEE. EEE as defined for this study is any deliberate and structured scheduled training. For an athlete the scheduled training sessions occur throughout a season at varying intensities, duration, and frequency. Together these factors influence the amount of energy expended for a single training bout. The 3 components and their contributions to total daily energy expenditure (TDEE) are shown in Figure 2.2. REE makes up over half of the energy expended in a day. EEE accounts for ~ 15 – 30% of the TDEE for a female athlete and the thermic effect of feeding represents ~ 10%.[72]

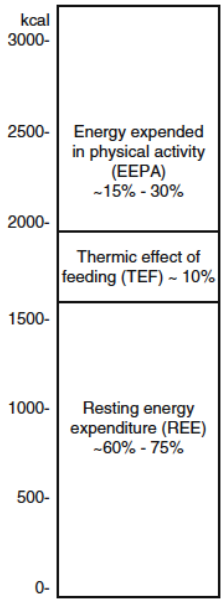


Figure 2.2. Components of TDEE

The high cost of DLW makes it of limited use in sport. In addition, DLW technique is unable to partition the different components of TDEE. Indirect calorimetry, the measurement of respiratory gas exchange to estimate metabolic rate, has limited use in the field settings due to cost and equipment. Although newer portable indirect calorimetry devices have been developed, Metamax™, Csomed K4b^2™, Aerosport™, additional testing is required to test the accuracy across a greater range of activities and environmental conditions.[73] Other methods used to estimate EEE include heart rate, questionnaires, activity recall, activity records, motion sensors, and combined methods (e.g. motion sensors and heart rate).[73] Newer items such as global positioning systems technology, near-infrared spectroscopy, and portable patches also exist but use in sport is limited. SenseWear Pro3 armband, (a portable electronic device that synchronically measures biaxial accelerometry, body heat loss, and galvanic skin response), seemed promising for the use in sport but a validation study did not provide accurate estimates of total or EEE in male endurance athletes compared to doubly labeled water due to the underestimation of energy expenditure at the higher exercise intensities.[93] Similar to EI, the method used to determine EEE should be selected based on the appropriateness in context of the research, taking into consideration the number of participants, cost and convenience.[73] Some methods are well-suited to particular sports e.g. portable indirect calorimetry devices or heart rate with motion sensors for running or biking. Where water sports may need to employ other methods suited for the sporting environment. Methods already used within certain sports create an opportunity to estimate EEE regularly with high compliance since the method is already in place. Written records or journals can provide detailed accounts of each individual's EEE over relatively short periods (i.e. 7 days) and become a suitable method when measuring different sports.[74]

Physical activity is quantified and described in a variety of ways. It can be described by type, intensity, frequency, and duration and can be simply categorized into occupational or leisure, continuous or intermittent, weight-bearing or non-weight-bearing.[74] Physical activity has also been quantified through using metabolic equivalents (METS) where 1 MET equals 3.5 ml O_2/kg/min and 1 kcal/kg/h, derived from the resting VO_2 of an ~70 kg, 40 year old male.[75] The premise of MET calculations is that progressively more vigorous activity requires a proportional rise in oxygen consumption that can be expressed through multiples of the 1 MET resting oxygen consumption value.[74] Although the MET value has been widely used as a method to determine energy expenditure, few validation studies against DLW have been completed since its conception in 1993. It has been validated as a method to calculate EEE in male endurance athletes for running and cycling.[76] Koehler et al, used standardized activity records comprised of a list of 25 pre-coded activities where the subjects documented activity time.[76] Energy expenditure was determined using a corrected MET value where the Cunningham equation was used to calculated each athlete's REE value.[76] This equation was used because of previous findings demonstrating its accuracy for determining REE in endurance athletes.[76] Indirect calorimetry was used to validate the MET values. For EEE, the activity records showed acceptable validity correlations with the indirect calorimetry for treadmill running (r=0.89, error SEE = 1.6 kcal/min) and for stationary cycling (r = 0.95, SEE = 1.4 kcal/min), and neither mean or proportional bias.[76] Even though no improvement in validity was shown through using the corrected MET value in this study, other research has shown that correction for the originally assigned resting VO_2 value of 3.5 ml/kg BW/min may have merit.[75,92] Both Bryne and Kozey found their subjects' resting oxygen consumption to be below 3.5 ml/kg/min.[75,92] Possibly no improvement was seen in the Koehler et al study as the study group were similar to the reference

value (determined in a 70kg, 40 year old healthy male). By using a predicted resting value as a correction for 1 MET, both Bryne and Kozey groups found a reduction in the error in the estimated energy cost for the activities measured.[75,92] Byrne et al, determined the magnitude of the variance in using the MET against indirect calorimetry in estimating energy expenditure across a large group of subjects consisting of 642 women, 127 men, 18-74 yr of age, 35 – 186 kg who were weight stable and otherwise classified as healthy.[75] The resting VO_2 was on average 2.6 ± 0.4 ml O_2/kg/min, only 14 (2%) of the 769 subjects tested had a value ≥ 3.5 ml O_2/kg/min.[75] The VO_2 values were significantly related to sex, BMI, age, percent body fat, waist circumference, fat mass and fat free mass. Using multiple regression analysis fat mass was the strongest predictor of the variability in resting VO_2 explaining 59% of the variance. Fat free mass, age, and gender significantly explained a further 1.9, 0.8, and 0.5%, respectively.[75] The suggestion of using a correction factor determined by a measured or predicted REE for a subject can help to adjust for individual differences.[75]

In addition, MET values are limited in the ability to capture an individual's current level of fitness or the adaptability of the body to physical activity.[74] An absolute exercise intensity of 10 kcal/min might be a warm-up for a trained individual but require immense effort for someone just starting an exercise program.[78] This relative intensity for aerobic intensity has been described in terms of percentages of VO_2max and has been adapted to reflect the absolute MET values.[78] The MET values for female athletes in this study can be corrected using their measured REE via indirect calorimetry and expressed in kg body weight.

Body Composition

It is more appropriate to express EA per FFM, as FFM is the metabolically active tissue in the body. Therefore an accurate method of measuring FFM is required.

The body is comprised of water, protein, minerals and fat. Body mass can be divided into fat mass and FFM (protein, minerals, and water). Various models have been created to assess these compartments. The 2-component models simply separate the body into fat mass and FFM. These models are based on body density (Db) using hydrodensitometry (HD). Several assumptions are made regarding the FM and FFM compartments; the densities of the 2 components (FM is 0.901 g/cc, FFM is 1.100 g/cc) are the same for all individuals and these densities are constant within an individual.[80] It has been shown that FFM varies across individuals and different populations. Additional population-specific two-component model equations have been developed to account for some of these differences.[81]

Advances in technology have developed 'gold standard' methods to determine the individual components of FFM (water, protein and mineral). Certain methods used to determine body composition provide reasonable accuracy for other variables; HD for estimating Db, hydrometry by isotope dilution for determining total body water, and DXA for assessing total body bone mineral. A combination of these methods increases the accuracy for assessing body composition. The use of individual reference methods reduces this accuracy, and field methods are least accurate. Table 2.3 provides a summary of this information in comparison to a 6-compartment chemical model.

Table 2.3. Accuracy level of methods measuring body composition compared to a 6-compartment chemical model[81]

Accuracy Level	**Method**	**Technical error (kg of fat)**	**Coefficient of reliability (%)**	**Limits of agreement (kg of fat)**
Most Accurate	4-C and 3-C methods that include TBW measure	< 0.8	≥ 99.5	< 1.1
Accurate	Individual reference methods (HD, ADP, hydrometry, DXA)	1-2	97 - 99	1.0 – 2.5
Least Accurate	Field methods (SKF, anthropometry, BIA, NIR)	2-4	85 - 95	2.5 – 4.0

For measuring body composition in athletes researchers have noted some important differences to consider before determining which model or method to employ. Because athletes have greater bone mineral content, bone mineral density, and fat free mass than non-athletes, the FFM density may be either higher or lower than the reference value of 1.100 g/cc.[81] Reasons for this include a training-dependent aspect (i.e. athletes involved in high-intensity, explosive training having a greater mineral to FFM ratio and thus a greater FFM density, i.e. athletes training for muscular hypertrophy having a greater water to FFM ratio and a lower FFM density).[81] Since the 2-component models are based on Db, and FFM density value is inconsistent when compared to the reference values used in the equations, the DXA method is preferable over the HD method for assessing athletes. In addition, since an increased bone mineral density in athletes also affects the FFM density, the DXA provides an accurate measure for total body bone mineral.[81] The DXA method is also preferable in the female athlete group to access bone mineral density due to the effect menstrual status and energy availability play in maintaining bone health.

Menstrual Status and the Oral Contraceptive Pill (OCP)

The appropriate classification of menstrual status in athletes requires additional measurements beyond a self-report. The analysis of blood or urine requires frequent and lengthy collection periods and imposes a high participant burden. Although these methods have been paramount in distinguishing menstrual status disturbances for practical settings less invasive and convenient tools are needed. While using self-report alone is not enough to discern normal menstrual function if used in conjunction with ovulation detection methods the likelihood of capturing a disorder increases. OCP use reduces the requirement for many of these measures since OCP suppress ovulation. The addition of exogenous sex steroids (estrogen and progesterone) also diminishes the ability to classify females based on hormonal cycling across menstruation

depending on how each individual responds to the dose and how this response is translated into endogenous secretion. A researcher is capable of initiating testing and data collection of subjects on consistent days where the exogenous hormone amounts from the OCP are constant. The consideration of the half-life of the exogenous hormones is relevant as well. Ethinylestradiol is detectable for up to 2 days after discontinuation, while some progestins are detectable for up to 5 days.[49] To decrease the burden for both researcher and participants using a combined 21 day OCP with a 7 day hormone free period removes the variability of determining the best days to initiating testing due to the half-life of the 2 exogenous hormones. Subjects can commence testing on day 1 – 4 of the pill cycle decreasing the factors from endogenous hormone levels.

The decision to use the above methods for determining EA in regularly menstruating trained females is evidenced-based and supports the goals of this study. The use of food and activity records to assess EI and EE will capture these values at an individual level while commencing data collection between days 1 – 4 of the pill cycle of the subjects will standardize testing dates.

At this time EA discrepancies remain between the current recommendations and the reported values. Whether these differences occur because of misreporting of energy intakes, overestimated recommendations, misclassification of menstrual status, differences between the laboratory findings in practical settings or explanations yet to be uncovered needs further exploration. In addition, how does the female athlete taking OCP navigate her prescription for health and performance? The combined approach of convenient field methods to estimate the EA of female athletes on OCP may increase the use of this concept in practical situations and determine its benefits to female athletes.

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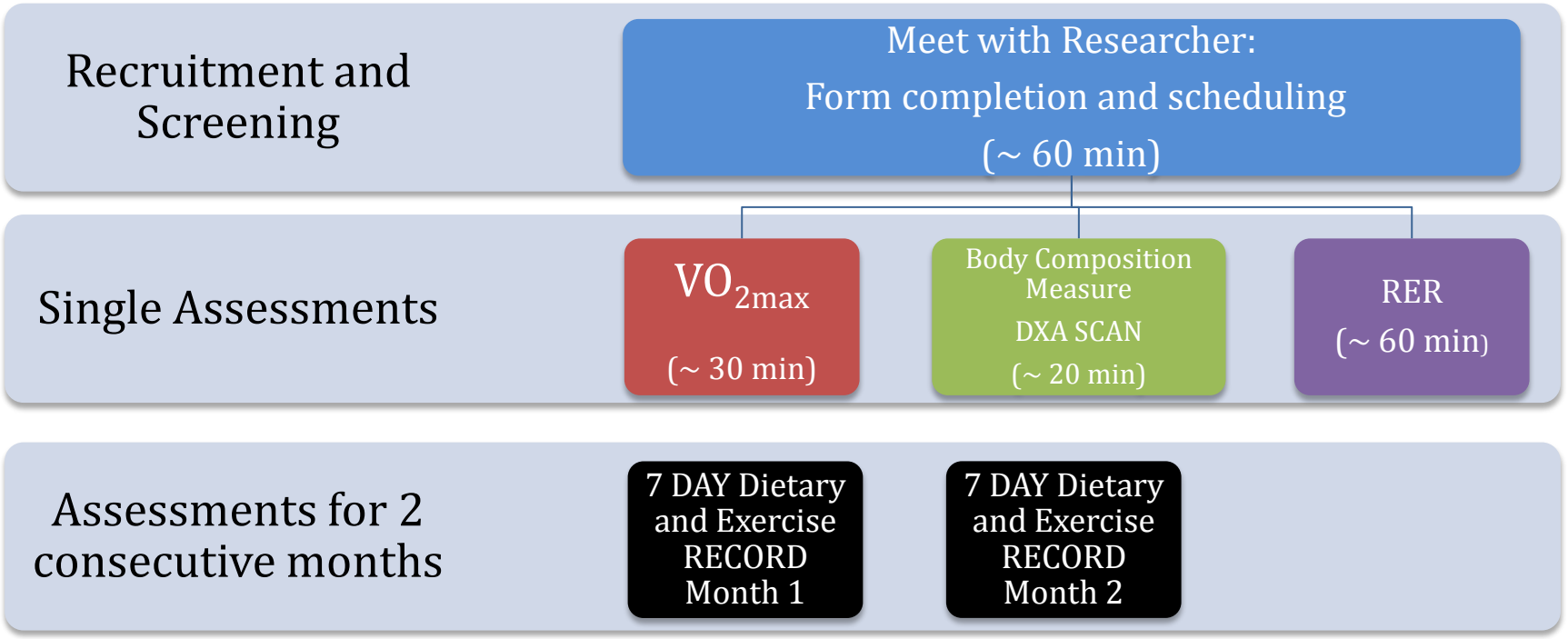
CHAPTER 3
METHODS

Experimental Design

This cross sectional study examined two groups of female participants across a 2 month period between March and November, 2011, assessing food intake, EEE, and FFM to estimate the EA of each individual. One group consisted of sedentary, healthy female participants taking OCP while the other group included female athletes taking OCP. Initial recruitment procedures screened participants for inclusion in the study, after which they continued with testing consisting of a DXA scan to measure FFM; completion of the Eating Disorder Inventory self-report (EDI) questionnaire; Dutch Eating Behavior Questionnaire – Restraint Scale (DEBQ-R); height; weight; REE, and aerobic fitness (VO_2max) testing. Each participant was asked to simultaneously record a 7-day food intake record and activity log for two consecutive pill cycles. The recording was completed in the follicular phase of the cycles initiated between the days of 1 - 4 of each individuals cycle based on pill count.

Table 3.0 Study Flow Chart

*All testing completed on University of Alberta campus



Recruitment

Participants were recruited through the use of posters placed around the University of Alberta campus. Emails to coaches of varsity teams were sent and meetings set up to explain the study to female athletes. Emails to provincial sport organizations and local sport clubs were distributed to recruit athletes. Non-athlete females were also recruited through posters placed around the University of Alberta Campus and emails to student associations in the health science, nursing, agriculture and nutrition faculties.

Inclusion Criteria

Initially only monophasic OCP were included in the study. The minimum time required to have been taking their current OCP brand was 3 months. Over the course of recruitment additional OCP formats were accepted. The OCP criteria is shown below:

a) Combined monophasic or triphasic brand
b) low dose estrogen (ethinylestradiol) content (value ≤ 0.035 mg)
c) 28 day pill cycle
d) low androgenicity (value of ≤ 1.0)

Initial Screening

Email contact from interested participants to the primary researcher was the initial form of contact to discuss entry criteria. The email informed the participants of additional study details and asked the participants to complete a questionnaire ensuring all criteria was met before meeting. Inclusion criteria for both groups included: 18 – 35 years of age, weight stable (± 2.2kg) for 3 months prior and good health as determined by self-report, nulliparous, no reported history of eating disorder or depressive illness within the past 3 years, same brand of OCP used for a minimum of 3 months that fulfilled the study's criteria. Participants reporting hypertension, diabetes, smoking, pregnancy, pituitary tumor, thyroid disorder, signs of perimenopause,

menopause, history or diagnosis of polycystic ovarian syndrome were excluded. Medication was not permitted with exception of the OCP.

The athlete participants were required to meet 2 of the following 3 criteria:

a. Competed provincial, national and/or international level

b. VO_2max of > 50 ml/kg/min

c. Structured training > 10 hours / week

The non-athletes did not exceed structured physical activity beyond 3 hours/week and were required to have a VO2max ≤ 45 ml/kg/min.

Ethics approval was granted from the University of Alberta Human Ethics Research Board. Written informed consent was obtained from each subject following a complete description of the study design provided both verbally and in written form.

At the initial meeting participants completed and signed the following forms for the study: DXA scan consent form and PAR-Q. They were provided a copy of the DXA scan information sheet. As well, paticipants completed the EDI and DEBQ-R questionnaire (Appendix H). The principal investigator provided verbal instructions and information sheets on how to complete the dietary food and activity records. These instructions were also included in written form within the recording booklets. Dates for the participants' recording were determined at the initial meeting based on each participant's pill cycle. Prior starting the actual dietary food and activity records, all participants completed a practice day of recording dietary food and activity and received feedback on their recording to ensure understanding of instructions and protocols as well as to improve accuracy of the task. Some participants preferred to complete the recording on the computer so an electronic copy of the booklets was provided.

Baseline Testing

Baseline testing included a VO_2max, REE and a DXA scan completed through the Human Nutrition Research Unit.

VO_2max Assessment

Aerobic fitness was determined to classify the athlete group and distinguish aerobic differences between athlete and non-athlete groups. This was assessed by a maximal oxygen consumption (VO_2max) test using a treadmill and a Parvo Medic's TureOne® 2400 metabolic cart. Testing was conducted in the Sport and Health Assessment Centre (Physical Education building) at the University of Alberta. This test was not scheduled in any particular time of the pill cycle phase. Gas and flow calibration was completed at the beginning of every test. The metabolic cart was calibrated against a reference mixture of oxygen and carbon dioxide gas. The test protocol was a progressive increase in treadmill speed and incline until the subject was no longer able to continue. Individualized protocols were used in accordance to ACSM recommendations.[1] Participants were oriented to the treadmill and warmed up at a comfortable jogging pace for about 5 minutes. The test began with a speed the participants felt comfortable at that typically equated to their warm-up speed. Speed was increased by 0.5 mph every 2 minutes until the participant reached ventilatory threshold (i.e. when the ratio of amount of air breathed to the amount of CO_2 produced reaches a nadir prior to a sustained increase of effort i.e. RER value $\geq$ 1.0 determined visually on the computer in real time display). At this point speed remained constant and the grade was increased by 2% every minute until the participant could no longer continue. Heart rate was monitored every minute using a Polar® heart rate monitor. Indication that VO_2max had been achieved was when a plateau of oxygen uptake or peaking over in oxygen uptake occurred. Secondary considerations for this were a respiratory exchange ratio of > 1.15

and whether the participants age-predicted maximum heart rate was recorded within 5 beats.[2] Measurements were expressed in absolute aerobic power (LO_2 /min) and relative aerobic power (ml O_2/kg/min).

REE Assessment

All REE testing was completed in the Women's Health and Physical Activity Laboratory in the Physical Education Building at the University of Alberta. REE was measured by indirect calorimetry using a Parvo Medic's TureOne® 2400 metabolic cart. Indirect calorimetry is the quantification of REE based on the gas exchange measurements of oxygen consumption (VO_2) and carbon dioxide production (VCO_2).[3] VO_2 and VCO_2 are calculated using a gas analyzer. Gas and flow calibration were completed at the beginning of every test. The metabolic cart was calibrated against a reference mixture of oxygen and carbon dioxide gas (Appendix G REE set up protocol). The room setting was quiet with low lighting. The room temperature was maintained at 20 – 25 ^{o}C.[4] Each participant's weight, height, sex, and age was entered into the software program prior the test. All REE testing was scheduled in the morning with start times ranging between 6:30 – 9:00 am, between Day 1 – 11 of the pill cycle. Participants were required to ensure the following prior testing: a) arrived to test by motor vehicle, not walking, bicycling or running b) arrived in a fasted state for a minimum of 12 hours c) arrived with no ingestion of caffeinated beverages for a minimum of 12 hours c) abstained from exercise (aerobic and resistance) for a minimum of 14 hours prior test.[4] On the testing day participants were asked if they had complied to the above. Participants were instructed to lie in a supine position for a resting period of 20 minutes. The initiation of testing commenced after this time. A transparent hood was placed over the participant's head and dilution pump was turned on. A total test time of 30 minutes was recorded for each participant once the flow rate of the dilution pump

maintained a diluted CO_2 percentage between 1 – 1.2% (dilution pump flow rate was between 15 – 30 L/min for all participants). The initial 5 minutes of testing was not included in total time. During testing participants remained in a supine position, awake and motionless.

VO_2 and VCO_2 were expressed in ml/min. To determine each participants 1 MET resting value (where a MET unit of measure is expressed as ml of O_2 consumption/kg body weight/min) the VO_2 ml/min as divided by body weight. To convert these values into energy expenditure (kcal/day) the Weir equation was used: M* = (3.941 x VO_2 ml/min) + (1.106 VCO_2 ml/min) x 1.41, and further divided by body weight to express in relative units.

*metabolic rate in kcal/day

Body Composition Assessment

Body composition was determined from a whole body scan using Dual Energy X-ray Absorptiometry (DXA) (General Electric LUNAR Prodigy High Speed Digital Fan Beam X-Ray-Based Bone Densitometer) located in the Li Ka Shing Centre for Health Research Innovation in the Human Nutrition Research Unit at the University of Alberta. The same certified medical X-ray technologist performed all scans. Most participants had their scans completed within Days 1-7 of the pill cycle in conjunction with 7-day assessment period. Because the technologist's schedule was set for only one day of the week and sometimes bimonthly, two participants were tested outside of this 7 day period but both fell on day 12 of the pill cycle. The same set protocol was followed for each participant. Participants and researchers were provided an information sheet that included the following:

- Patient ID #, age, sex, race, height, weight, BMI
- Date, time, technologist, software version, historical trends

- Whole body and regional absolute values for: fat (g), lean (g), BMC (g), BMD (g/cm^2), area (cm^2), total tissue mass (kg)
- Whole body fat free mass (g)
- Whole body and regional: tissue % fat, region % fat
- Z-score and graph for: tissue % fat
- Z-score, T-score and graph for BMD

Participants received their scan results once the investigator collected all dietary food and activity records.

Body Mass Measurement

Weight was measured at the beginning of the REE, VO_2max, and DXA scan tests. The weight used for EEE calculations was the one obtained from the REE test because all subjects were in a fasted state (Weight Watchers® Digital Glass scale). Participants recorded their body weight on Day 1 and Day 7 of the EI and EEE recording periods.

Height Measurement

The height measurement was obtained during the body composition assessment using a Quick Medical Heightronic digital stadiometer 235 (Northbend, WA) to the nearest 0.1-inch. The Medical X-ray technician obtained the measurement used.

Eating Disorder Inventory (EDI) and Dutch Eating Behavior Questionnaire – Restraint Scale (DEBQ-R)

The EDI is a validated questionnaire that assesses eating attitudes and behaviors (Appendix H).[5] This self-report 64 item questionnaire is comprised of 8 scores divided into subscales to measure multidimensional symptom clusters commonly related to eating disorders. The subscales are Drive for Thinness, Bulimia, Body Dissatisfaction, Ineffectiveness, Perfectionism, Interpersonal

Distrust, Interoceptive Awareness, and Maturity Fears.[5] Individuals are asked to response based on a 6 point scale ranging from "always" to "never" (always, usually, often, sometimes, rarely, never). Responses are assigned a value of 0 – 3 where the upper or bottom three responses are all weighted 0 depending on scoring of the question. For example, for negatively scored questions, "always", "usually" and "often" responses would be equated 0 and "sometimes" would be given a value of 1, "rarely" a value of 2 and "never" a value of 3.[5] The questionnaire is commonly used in athletic populations and has shown good internal consistency.[6]

The DEBQ-R assesses restrained eating behaviors (Appendix H; Questions 65 – 74 on the questionnaire).[7] It consists of 10 questions used to discern eating behaviors related to deliberate planned weight control. Responses range from "very often" to "never" (very often, often, sometimes, seldom, and never). The scoring is on a 5 point value and is summative. The response "very often" equates to 5 and the values decrease by 1 unit to "never". Two questions have an option of a "not relevant" response and scored accordingly in the analysis.[7] It has also demonstrated good internal consistency.[8]

Month 1 and 2 Food and Activity Records

Dietary food and activity records were collected for 2 separate 7-day periods over 2 consecutive pill cycles. All participants initiated the 7-day assessment between Day 1 – 4 of the pill cycle and were completed between Day 7 – 11. Each participant completed 14 days of dietary food and activity records.

Energy Intake (EI)

EI was assessed using a 7-day intake prospective food record initiated between Day 1- 4 of the pill cycle. The dietary food records were analyzed using computerized nutrient analysis software (Nutribase 9 SE Pro by Cybersoft Inc.). To reduce recording and inputting errors the following

were incorporated into this study: a) having all subjects complete a practice day to receive feedback on how well they recorded their daily intake and to ensure full understanding of recording daily intake b) having only 1 data entry person who was experienced and knowledgeable in sport products and foods in Alberta c) a protocol for quality control of the processing of each food record was employed, and in this case each participant's food record was reviewed three times to ensure all foods were entered correctly and standardized for the same item for multiple entries. Food products distinct to the Edmonton and surrounding region were entered as separate food items into the database and repeated as necessary to ensure highest accuracy. In addition, most participants provided specific food labels and product web links of consumed items to ensure that the most detailed and accurate nutrient analysis could be conducted. Participants recorded all beverages, including water amounts, and specific supplement information. Supplements included protein powders, sport drink beverages, multivitamin mineral complexes and individual nutrient supplementation. Herbal supplements were not included, as the Nutribase program does not include these in the analysis. The same person entered and analyzed all dietary record data. Intakes were analyzed for total calorie intake and macronutrient breakdown. Specific sport recommendations for carbohydrate (CHO) and protein have been established.[1] The specific requirements are dependent on a variety of factors and are influenced by the energy systems employed by the demand of the sport, training schedule, level of the athlete, and specific athlete performance goals. In general the athlete recommendations for the above nutrients differ from those of sedentary individuals. Averages of micronutrients were also determined. Only calcium, iron, and vitamin D were included in the statistical analysis. Calcium and iron are minerals often low in athletes with menstrual dysfunction and low energy intakes potentially impacting physiological health.[11,12] Vitamin D

dietary reference intakes have recently been increased from the amount of 200 IU /day to an amount of 600 IU /day.[13] Health Canada statistics on vitamin D values collected between 2007 and 2009 indicated a mean serum value below the optimal range.[13] The inclusion to assess vitamin D intake was included to observe the intakes of the participants in light of the new DRI. Participants were asked to include all supplements taken, i.e. vitamins, mineral, multi preparations, protein powders, etc. And although vitamins and minerals do not add energy to the subjects' diet, the use of various supplements was included in the analysis for participant information. See Appendix E for food intake forms.

Exercise Energy Expenditure (EEE)

Activity record sheets were given to participants to document deliberate engagement of a structured activity or training session(s) during a day and starting on the same day as the energy intake records and were completed for the same 7 days. Type, duration and intensity (RPE or HR monitor or watts or speed) of activity were recorded. Ainsworth et al, compendium of physical activity, online version, http://sites.google.com/site/compendiumofphysicalactivities/, was used to determine MET worth of recorded activities.[9] Measured REE values for each subject were used to apply a correction factor for a 1 MET value since the MET value is based on a preset value of 3.5 mlO_2/kg/min.[10]

To convert kcal/day obtained from the REE testing to ml/kg/min the following equation was used:

(3.5 ml/kg/min (REE kcal/day / 1440 / 5 / weight in kg)) x 1000

Tables 3.1 and 3.2 outline the differences that occur with these adjustments. An example using the MET value of running at 5 mph has been presented. Table 3.1 shows without correction (using measured REE for the subjects), both have slightly greater exercise expenditure. If the

participants were to have the same weight (Table 3.2) the athlete has a lower expenditure if the measured REE values are used. If the measurement is only based on body weight the expenditure is the same for both participants.

Table 3.1. MET Calculation without a correction factor

EEE (kcal) = weight (kg) x MET (kcal/kg/h) x duration (h)

Participant	Measured REE(ml/kg/min)	Weight (kg)	MET: Activity Running 5 mph	Duration: 30min	Corrected MET (kcal/kg/h)	Corrected EEE kcal	No correction EEE kcal
Athlete	3.7	58	8	0.5 h	(3.5/3.7) x 8 0.95x8 = 7.5	58x7.5x0.5 = 219	58x8x0.5 = 232
Non-athlete	3.0	62	8	0.5 h	(3.5/3.0) x 8 1.17x8 = 9.3	62x9.3x0.5 = 289	62x8x0.5 = 248

Table 3.2. MET calculation using correction factor when participants have the same weight

EEE (kcal) = weight (kg) x MET (kcal/kg/h) x time (h)

Participant	Measured REE (ml/kg/min)	Weight (kg)	MET: Activity Running at 5 mph	Duration: 30 min	Corrected MET (kcal/kg/h)	Corrected EEE kcal	No correction EEE kcal
Athlete	3.7	58	8	0.5 h	0.946 x 8	219	232
Non-athlete	3.0	58	8	0.5 h	1.17 x 8	270	232

Energy Availability calculations

EA is defined by the following equation:

EA = EI – EEE or EA = (EI-EEE) / FFM

Where both EI and EEE can be expressed as an amount of kcal/day and also in relative terms of kcal/kg FFM/day. EA was expressed as kcal/kg FFM/ day. For example, if a food record collected for participant A is calculated to be 2500 kcal/day and the FFM of participant A is 45 kg, this would equate to a relative EI of 55.6 kcal/kg FFM/day. If the EEE log for this participant

is 800 kcal / day it would equate to 17 kcal/kg FFM/day. EA would therefore be calculated at 55.6 – 17.8 kcal/kg FFM/day = 37.8 kcal/kg FFM/day. EA values were calculated daily and summed weekly for each participant. Comparison between groups was based on the weekly average. Each week was analyzed independently.

Statistical Calculations

Independent t tests were conducted to determine mean group differences between the athlete and non-athlete group in demographics, aerobic assessment, EDI and DEBQ-R questionnaires, energy intake, exercise energy expenditure, and energy availability. Pearson's correlation coefficient analyses were completed on the EDI subscales associated with weight and diet concerns (i.e. drive for thinness, bulimia, and body dissatisfaction)[14] and DEBQ-R scores. Data are reported as means ± SD, including range values. Differences of $p < 0.05$ were considered statistically significant. Data were analyzed using IBM® SPSS® for Mac (version 20).

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CHAPTER 4
RESULTS

Demographic Characteristics

A total of 23 healthy females completed the study protocol (athlete n=12, non-athletes n=11). The demographic characteristics of the participants are presented in Table 4.0. The athlete and non-athlete groups were not different in age, age of menarche, gynecological age, weight, and body mass index (BMI). The difference between height was statistically significant ($p = 0.022$) with the athletes being taller. The athlete group had a significantly lower percent body fat ($p < 0.001$) and fat mass ($p = 0.05$) and significantly higher fat free mass (FFM), ($p < 0.001$) than the non-athlete group. Whole body bone mineral density differed significantly between groups ($p = 0.020$); with the athlete group have higher values. The body mass measurements taken by the participants during the recording periods of EI and EEE were not different from the measurements taken by the researcher across the study period.

Aerobic Assessment

Both absolute and relative values for VO_2max were significantly higher for the athlete group ($p < 0.001$). The Bruce Treadmill Ramp Protocol was used for one athlete due to a knee injury. The athlete had already met 2 of the 3 criteria for inclusion to the athlete group and the results of the maximal aerobic test would not eliminate her from the study. Her relative result was 45.6 ml/kg/min and absolute 3.14 L/min. The results for two potential non-athlete participants for the maximal aerobic assessment were too high to meet the inclusion criteria for that group (values were 45.5 and 47.1 ml/kg/min). Upon review of their weekly activity levels they were participating in regular structured activity and they were excluded from the study.

Table 4.0. Characteristics of athletes and non-athletes

Variable	Non-athlete **(n = 11)**	**Range Values**	Athlete **(n = 12)**	**Range Values**	P value
Calendar Age (yr)	25.1 ± 4.0	20 – 31	23.8 ± 3.3	20 - 32	0.392
Age of Menarche (yr)	12.7 ± 1.6	10 - 16	13.4 ± 1.9	10 – 16	0.330
Gynecological Age (yr)	12.5 ± 4.7	4 - 21	11.1 ± 4.1	5 - 18	0.464
Height (cm)	1.63 ± 0.07	1.50 – 1.73	1.70 ± 0.07 *	1.52 – 1.78	0.022
Weight (kg)	60.6 ± 7.7	49.2 - 75	63.1 ± 6.2	52.9 – 72.6	0.388
BMI (kg/m^2)	22.9 ± 2.7	19.3 – 29.3	21.9 ± 2.2	18.9 – 25.0	0.339
Fat Mass (kg)	20.3 ± 5.3	14.7 – 29.7	13.6 ± 5.0 *	7.7 – 22.5	0.050
Fat Free Mass (kg)	39.2 ± 4.2	29.5 – 43.4	49.2 ± 4.8 *	38.3 – 58.0	<0.001
Bone Mineral Density (g/cm^2)	1.12 ± 0.05	1.06 – 1.20	1.18 ± 0.07 *	1.06 – 1.29	0.020
Regional Body Fat (%)	33.8 ± 5.5	25.8 – 41.0	21.3 ± 6.8 *	12.9 – 31.4	<0.001
Vo_{2max} (ml/kg/min)	37.18 ± 3.54	32.30 – 42.60	47.10 ± 4.17 *	40.90 – 54.20	<0.001
Vo_{2max} (l/min)	2.25 ± 0.28	1.57 ± 2.59	2.98 ± 0.37 *	2.50 ± 3.71	<0.001

Values are mean ± SD

* Significant difference of <0.05

Oral Contraceptive Pill Types

Table 4.1. provides the oral contraceptive pills taken by the participants. All the types of pills met the inclusion criteria. It was assumed that all participants maintained the regular use and consistency of the pill across both cycles. Ten athletes were taking monophasic OCP and the remaining 2 were on a triphasic brand. Eight non-athletes were taking monophasic and 3 were on triphasic OCP brands.

Table 4.1. OCP brands used by athletes and non-athletes

OCP Brand	Ethinylestradiol dose (mg)	Progestogen type dose (mg)	Androgenicity Value	OCP Type
Alesse (3)*	0.020	Levonorgestrel, 0.10	0.83	Monophasic
Diane 35 (1)*	0.035	Cyproterone, 0.20	NA	Monophasic
Loestrin 21 (1)*	0.020	Norethindrone, 1.0	1.0	Monophasic
Marvelon (4)*	0.030	Desogestrel, 0.15	0.51	Monophasic
Yasmin 21 (5)*	0.030	Drospirenone, 3	Antiandrogenic	Monophasic
Yaz (4)*	0.020	Drospirenone, 3	Antiandrogenic	Monophasic
Ortho Tri-cyclen (2)*	0.035	Norgestimate 0.18, 0.215, 0.25	0.48	Triphasic
Tri-cyclen Lo (1)*	0.025	Norgestimate 0.18, 0.215, 0.25	0.48	Triphasic
Triquilar (1)*	0.030	Levonorgestrel 0.05, 0.075, 0.125	1.0	Triphasic

***Number in brackets denotes number of subjects on particular brand**

The average length of time the athlete group was taking this same OCP brand was 3.8 years with a range of 8 months – 13 years. The non-athlete group's average length of time was 4 years with a range of 1.2 – 14 years.

EDI Scores and DEBQ-R Scores

There were no significant differences between the groups in any of the subscale traits outlined in the EDI questionnaire or the results from the DEBQ-R scores. Correlation values are presented

Table 4.2 EDI and DEBQ-R for athletes and non-athletes

EDI	Non-athlete (n = 11)	Range Values	Athlete (n = 12)	Range Values	P value
Drive For Thinness	2.5 ± 4.9	0 - 16	2.8 ± 4.0	0 - 14	0.840
Bulimia	1.1 ± 1.2	0 - 5	1.1 ± 1.6	0 - 3	0.942
Body Dissatisfaction	4.0 ± 3.3	0 - 11	2.4 ± 3.5	0 - 10	0.279
Ineffectiveness	0.6 ± 1.1	0 - 4	0.3 ± 1.2	0 - 3	0.531
Perfectionism	5.2 ± 4.2	1 - 15	4.8 ± 3.3	0 - 11	0.786
Interpersonal Distrust	0.3 ±0.6	0 - 2	0.8 ± 1.4	0 - 4	0.301
Interoceptive Awareness	0.5 ± 1.5	0 - 5	0.5 ± 1.4	0 - 5	0.942
Maturity Fears	1.1 ± 1.4	0 - 6	1.4 ± 1.9	0 - 4	0.649
DEBQ-R	Non-athlete (n = 11)	Range Values	Athlete (n = 12)	Range Values	P value
Dietary Restraint	2.2 ± 0.9	1.1 - 3.8	2.2 ± 0.7	1.2 - 3.7	0.900

Values are mean ± SD

in Appendix L. No significant correlations between the above subscale scores and dietary intake were detected.

Energy Status

"Energy Status" refers to the caloric variables that affect the participants' energy status and thus factor into the individual's energy availability (EA).

Resting Energy Expenditure

Although all participants were asked to arrive in a rested state some activity was unavoidable by some participants due to the mode of transportation to the University of Alberta. Table 4.3 provides the REE values for both groups. The group mean for the athletes was 1738 ± 150 kcal/day with a range of 1447 – 1937 kcal/day. This was significantly higher (p=0.006) than the non-athlete group, 1519 ± 192, range 1224 – 1880 kcal/day. When expressed per kg body weight a statistical difference remained between the groups (p=0.013).

Exercise Energy Expenditure

Both groups reported walking, biking to work and/or school on their activity log with no other daily routine activities recorded. The athlete group consisted of participants from the following sports: hockey (1), bouldering (1), triathlon (1), track and field (2), swimming (3), power lifting (2), volleyball (1), and rugby (1). The average exercise amount for the athlete group for week 1 was 520.3 ± 210.9 min/week (range of 160 – 750) and week 2, 629.7 ± 301.5 min/week (range 340 – 1340). The values for both weeks were significantly higher than the non-athlete group ($p<0.001$). All participants were asked to continue their regular schedule or habits and not modify anything during the course of the study.

Sample EEE calculations are provided in Appendix J using both non-corrected and the corrected MET value. However, all data analysis was completed using the corrected MET values based. EEE was significantly higher ($p<0.001$) for the athlete (543.5 ± 253.2 kcal/day week 1 and 547.5 ± 175.7 kcal/day week 2) than the non-athlete group (62.0 ± 55.6 kcal/day week 1 and 72.1 ± 64.2 kcal/day week 2) for both weeks.

Energy Intake

All participants returned 14 days of intake records. One participant was out of town during the second week of food recording and took pictures of all the food consumed and food labels to help with distinguishing portion sizing and food items for the researcher. No statistical significance was shown between the energy intakes of the groups when expressed as absolute (kcal/day) and relative (kcal/kg/day) values.

Table 4.3. Energy status of athletes and non-athletes

Variable	Non-athlete (n = 11)	Range Values	Athlete (n = 12)	Range Values	P value
EI (kcal/day) WK1	1972 ± 352	1459 - 2373	2176 ± 352	1555 - 2814	0.146
EI (kcal/day) WK2	1882 ± 168	1641 - 2119	2124 ± 478	1518 - 2948	0.122
EI (kcal/kg/day) WK1	32.7 ± 4.0	26.4 – 38.7	34.8 ± 6.9	22.3 – 46.7	0.373
EI (kcal/kg/day) WK2	31.5 ± 4.4	24.7 – 38.0	34.2 ± 8.2	21.6 – 45.9	0.324
EEE (kcal/day) WK1	62.0 ± 55.6	0 – 156.6	543.5 ± 253.2 *	181.0 – 1134.3	<0.001
EEE (kcal/day) WK2	70.1 ± 64.2	0 – 176.4	547.9 ± 175.7 *	358.8 – 979.1	<0.001
EEE (min/week) WK1	108.0 ± 92.6	0 – 255.0	520.3 ± 210.9 *	160.0 – 750.0	<0.001
EEE (min/week) WK2	115.6 ± 127.5	0 - 350	629.7 ± 301.5 *	340 - 1340	<0.001
REE (kcal/day)	1519 ± 192	1224 – 1880	1738 ± 150 *	1447 - 1937	0.006
REE (kcal/kg/day)	25.1 ± 1.9	23.3 – 28.6	27.7 ± 2.4 *	24.5 – 32.3	0.013
EA (kcal/day/FFM) WK 1	48.7 ± 6.1	38.5 – 58.2	33.1 ± 8.2 *	20.7 – 49.5	< 0.001
EA (kcal/day/FFM) WK 2	46.7 ± 7.3	38.2 – 60.30	31.5 ± 9.5 *	18.8 – 60.3	< 0.001

Values are mean ± SD
WK = week, EI = energy intake, EEE = exercise energy expenditure, REE, resting energy expenditure, EA = energy availability
* Significant difference of <0.05

Energy Availability

All participant values for EA are found in Appendix K. For week 1 and week 2 the EA values were significantly higher in the non-athlete versus the athlete group ($p<0.001$). The EA values for the non-athlete group were 48.7 ± 6.1 and 46.7 ± 7.3 kcal/kg FFM/day, week 1 and 2 respectively. For the athletes these values were 33.1 ± 8.2 and 31.5 ± 9.5 kcal/kg FFM/day. The ranges of EA for the non-athlete group were 38 – 58 and 38 – 60 kcal/kg FFM/day for week 1 and week 2 respectively. The EA range for the athlete group was ~21 – 50 for week 1 and ~19 – 60 for week 2 kcal/kg FFM/day. Individually, there were no non-athlete participants that fell near or below 30 kcal/kg FFM/day threshold for any average for either week. In contrast, only 2 athletes were above this threshold value for both weeks and were from the same sport (power lifting). The other athletes had either one or both weeks near or below this 30 kcal/kg FFM/day value. The three swimmers had values well below 30 kcal/kg FFM/day for both weeks.

Nutrients

Table 4.4 presents CHO, protein, and fat expressed in absolute and relative terms for the two groups. In addition, calcium, iron, and vitamin D have also been included. Differences between the athlete and non-athlete groups were not different for carbohydrate, fat, iron, and, and vitamin D. Calcium intake for week 1 was higher in the athlete group, 1197 ± 539 mg, range of 758 – 2801 mg compared to the non-athlete group, 739 ± 198 mg, range of 439 – 995 mg ($p = 0.015$). The relative values of CHO for both weeks were not significantly different between groups. The values for the athletes for week 1 and 2 were 4.9 and 4.5 g/kg/day, respectively. The ranges for each week were 2.6 – 7.3 and 2.2 – 6.1 g/kg/day. For week 1 the total amount of protein for athletes was significantly higher compared to the non-athletes but when expressed per kg body weight there was no longer a difference. Table 4.5 compares the athlete values of these nutrients

in reference to the recommended amounts for these nutrients based on the Dietary Reference Intakes (DRI) and specific sport nutrition recommendations.[1,2,3] Specifically, 75 and 95% of the athletes had below recommendations for vitamin D for week 1 and week 2, respectively. Athletes fell below the recommendations for iron 50% for week 1 and 75% for week 2. The percentage of reported intake of CHO for the athletes that were lower than the 6 g/day recommendations was 92 and 83% for week 1 and week 2 respectively.

Table 4.4. Specific nutrient values for athletes and non-athletes

Variable	Non-athlete (n = 11)	Range Values	Athlete (n = 12)	Range Values	P value
Carbohydrate g/day WK1	262 ± 42	204 - 339	304 ± 63	181 - 437	0.079
Carbohydrate g/day WK2	250 ± 22	218 - 292	282 ± 68	151 - 371	0.153
Carbohydrate g/kg /day WK1	4.4 ± 0.8	3.1 – 5.7	4.9 ± 1.1	2.6 – 7.3	0.250
Carbohydrate g/kg /day WK2	4.2 ± 0.7	3.4 – 5.4	4.5 ± 1.2	2.2 – 6.1	0.459
Protein g/day WK1	72 ± 14	53 - 102	88 ± 19 *	54 - 121	0.036
Protein g/day WK2	76 ± 7	63 - 87	93 ± 19	62 - 150	0.059
Protein g/kg /day WK1	1.2 ± 0.3	0.9 – 1.6	1.4 ± 0.4	1.0 – 2.3	0.143
Protein g/kg /day WK2	1.3 ± 0.2	1.0 – 1.7	1.5 ± 0.4	0.9 – 2.2	0.176
Fat g/day WK1	69 ± 21	40 - 107	71 ± 11	55 - 88	0.703
Fat g/day WK2	63 ± 14	51 - 89	72 ± 22	41 - 125	0.272
Iron (mg) WK1	13.2 ± 5.5	6 – 26	18.5 ± 9.5	10 – 41	0.119
Iron (mg) WK2	13.9 ± 4.9	9 – 25	14.7 ± 5.9	7 – 25	0.741
Calcium (mg) WK1	738.6 ± 198.4	439 – 995	1196.9 ± 538.9 *	758 – 2801	0.015
Calcium (mg) WK2	910.7 ± 474.3	423 – 2140	1126.6 ± 355.8	550 – 1750	0.228
Vitamin D (IU) WK1	157.6 ± 110.6	51 – 411	420.7 ± 420.1	63 – 1257	0.373
Vitamin D (IU) WK2	194.4 ± 137.3	40 – 446	244.7 ± 161.8	81 - 620	0.986

Values are mean ± SD
* Significant difference of <0.05

Table 4.5. Comparison of specific nutrients of athlete participants and DRI and recommendations

Variable	DRI or Sport Nutrition Recommendations	Athlete (n = 12)	Range Values	Participants consuming < % (n)
Carbohydrate g/kg/day WK1	Moderate Intensity (~1 hr/day) 5 -7 g/kg/day [a]	4.9 ± 1.1	2.6 – 7.3	< 5 = 42 (5)
Carbohydrate g/kg/day WK2	Endurance Program (1-3 hr/day) 6 – 10 g/kg/day [a]	4.5 ± 1.2	2.2 – 6.1	<6 = 92 (11) < 5 = 50 (6)
	Extreme Commitment (> 4-5 hr /day) 8 – 12 g /kg/day [a]			< 6 = 83 (10)
Protein g/kg WK1	1.2 – 1.7 g /kg/day [b]	1.4 ± 0.4	1.0 – 2.3	33 (4)
Protein g/kg WK2		1.5 ± 0.4	0.9 – 2.2	42 (5)
Fat % WK1	25 – 35% of daily calories [c]	30 ± 4	25 - 36	0 (0)
Fat % WK2		31 ± 7	21 - 43	17 (2)
Iron (mg) WK1	18 mg /day [c]	18.5 ± 9.5	10 – 41	50 (6)
Iron (mg) WK2		14.7 ± 5.9	7 – 25	75 (9)
Calcium (mg) WK1	1000 mg /day [c]	1196.9 ± 538.9	758 – 2801	42 (5)
Calcium (mg) WK2		1126.6 ± 355.8	550 – 1750	33 (4)
Vitamin D (IU) WK1	600 IU/day [c]	420.7 ± 420.1	63 – 1257	75 (9)
Vitamin D (IU) WK2		244.7 ± 161.8	81 - 620	92 (11)

Values are mean ± SD,
[a] Burke et al, 2011. [b] Position Stand: Nutrition and Sport Performance 2009. [c] DRI: http://www.nap.edu/topics.php?topic=380

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CHAPTER 5
DISCUSSION

EA of female athletes taking OCP

Current literature reporting EA of female athletes in a practical setting is sparse, but some studies have found that the EA for sedentary groups were significantly higher than the athlete groups.[1,2] Female athletes taking OCP have been largely excluded despite the prevalence of their use. To explore this comparison the EA of female athletes and non-athletes taking OCP was compared over 2 consecutive pill cycles for a period of 7 days for each cycle. It was hypothesized that the non-athlete group would have higher EA than the female athletes. This was confirmed where the present results showed significantly higher EA for the non-athlete group compared to the athletes across both weeks.

The mean EA for the athlete participants in this study for week 1 and week 2 were 33 and 31 kcal /kg FFM/day, respectively and are similar to the range reported by other research of 19 – 33 kcal/kg FFM/day for eumenorrheic athletes.[1] The only other EA value determined for athletes taking OCP was that by Thong et al.[6] This EA value of 33 kcal/kg FFM/day included other recreationally healthy eumenorrheic female athletes in the study. It appears that the healthy female athletes taking OCP have similar EA compared to eumenorrheic athletes. EA for sedentary groups previously determined in the literature have also found higher EA than the athlete groups. Sheid et al, determined the baseline values for EA in healthy participants in a range of 42 – 45 kcal/kg FFM/day.[4] In healthy exercising women, Reed et al, determined the EA to be 42 kcal/kg FFM/day.[5] For the two weeks of this study the EA of the sedentary group were 49 and 47 kcal/kg FFM/day, respectively. Amenorrheic athlete EA ranges have tended to be lower, 16 – 28 kcal/kg FFM/day, than both the eumenorrheic and sedentary groups.[1] This study

and Reed et al, included subjects performing a variety of sports while Thong et al, included aerobically based trained females (e.g. running, cycling, aerobics) and the other studies included runners, cyclists and triathletes.[1,5,6] A recent study measuring EA in dancers found the lowest values for both eumenorrheic and amenorrheic dancers, 5.8 and 0.6 kcal/kg FFM/day, respectively over a 3 day period.[2] Combining the above findings and including the EA data from the present study it seems that sedentary healthy females have higher EA than eumenorrheic athletes and healthy athletes taking OCP, who have higher EA than amenorrheic athletes. EI was not significantly different between the non-athlete and athlete groups in this study. Both FFM and EEE were significantly higher in the athlete group thus the lower EA. This could suggest that the athletes were unable to increase dietary intake to compensate for higher energy expenditure from exercise and FFM. The average EA for the athletes for both weeks was well below the current recommendations for weight maintenance of around 45 kcal/kg FFM/day.[1, 3] The estimated EA values were actually closer to the lowest end of the recommendations for weight loss (i.e. 30 kcal/kg FFM/day) for all but 2 of the athlete subjects.[3] Regardless of the actual numbers, these above findings indicate that the estimated EA values in the practical setting for female athletes are lower than expected and lower than what is currently recommended for EA in terms of weight maintenance and perhaps energy stability. Whether this deviation from the recommendations poses a health concern or detrimental effects to the athlete remains unclear. However, Reed et al, Thong et al, and De Souza et al, all used hormone verification to ensure normal menstruation for the groupings.[5,6,10] This may suggest that lower than recommended EA for female athletes may pose no risk to menstrual health. Possibly athletes habituate to training making the energy requirement or physical demands less than otherwise calculated. It remains

difficult to determine why this discrepancy continues to present with these groups of female athletes.

Determining energy intake and exercise energy expenditure

To assess EA this study used prospective dietary intake booklets and exercise logs as noninvasive tools to measure EI and EEE. These tools have previously been used in the research estimating EA and other studies examining EI and energy expenditure independently. Albeit very sophisticated methods and technology pieces exist for some of these measurements, the financial burden, space allocation, and application variance still creates usage barriers.

Energy Intake

The mean 7-day estimated absolute EI values were not significantly different between the athlete and non-athlete groups across both weeks (athlete week 1, 2176 ± 352 and week 2, 2124 ± 478; non-athlete week 1, 1972 ± 352 and week 2, 1882 ± 168 kcal/day). These findings are similar to previously reported EI comparisons of healthy athlete and non-athlete groups that are also not statistically different.[6,10,11,12,13] Reported comparisons of EI between eumenorrheic and amenorrheic athletes are inconclusive; some showing no significant difference[5,11,16], others reporting amenorrheic athletes having lower EI than eumenorrheic athletes[2,6,10,14,15], and one reported EI of amenorrheic athletes greater than eumenorrheic athletes[7]. When expressed per kg FFM in the current study no significant difference remained. However, when Reed et al expressed the EI per kg FFM, a significantly lower EI in the exercising menstrual dysfunction group compared to the regularly ovulating group was detected.[5] Regarding athletes, expressing EI per kg FFM should be adopted since a greater FFM would further decrease the available energy for other body processes.

Exercise Energy Expenditure

Using the corrected MET method the EEE for the current study for the athlete group for week 1 was 544 ± 253 (range of 181 – 1134 kcal/day) and 548 ± 176 (range of 359 – 979 kcal/day) for week 2. The non-athlete group EEE values were: 62 ±55.6 (range of 0 – 157 kcal/day) for week 1 and 70 ± 64.2 (range of 0 – 176 kcal/day) for week 2. Although other studies have used different methods for recording and obtaining EEE, the current findings are similar to ranges reported in the literature. Dolye-Lucas et al, also used the MET calculations but used a standard MET value of 4.8 for all the dancers over a standard practice time, (EEE for eumenorrheic dancers = ~1305 kcal/day and ~266 kcal/day for control group).[2] Thong et al, had subjects complete a prospective 7 day exercise diary and used energy expenditure tables to estimated average EEE (aerobically active females taking OCP = 579.9 ± 59.6, elite eumenorrheic athlete = 954.6 ± 54.7, elite amenorrheic athlete = 970 ± 32 kcal/day).[6] A combination of methods was used by Reed et al, for determining EEE of healthy active females. They provided the subjects with HR monitors with a built in calorie-computing feature. Purposeful exercise was defined as sessions longer than 10 minutes with a HR above 90 beats /min.[5] When subjects were unable to wear the HR monitor (e.g. swimming) EEE was calculated using METS (EEE of ovulating group = 296.4 ± 255 range of 22 – 771 kcal/day and exercising associated menstrual disturbance group 405 ± 222.7 range of 135 – 965 kcal/day).[5] Hoch et al, used accelerometers that had been individually calibrated for each subject at 2 self-selected exercise intensities and worn continuously for 72 hours. The energy expended while wearing the accelerometer was used to calculate EA so it is unclear whether the actual exercise expenditure could have been partitioned from the total amount (values were not provided).[20] De Souza et al, measured both total 24 hour energy expenditure using Caltrac accelerometers and EEE with analysis of training activity

records (EEE for sedentary group = 0, ovulating exercising group = 23.3 ± 1.6, anovulating exercising group = 272 ± 78.3 kcal/day).[10] Laughlin et al, used energy expenditure tables to estimate EEE in eumenorrheic athletes (906 ± 68 kcal/day) , amenorrheic athletes (1074 ± 106 kcal/day) and sedentary controls 62 ± 19 kcal/day).[7] Tomten et al, also investigating energy balance, found the EEE of eumenorrheic and amenorrheic runners to be 501 ± 71.7 and 525 ± 95.5 kcal/day, respectively.[14] Manore et al, provides a summary of EEE for athletes with varying menstrual status, and the range for eumenorrheic and amenorrheic athletes was 402 – 906 and 476 – 1107 kcal/day, respectively.[1] For healthy control groups the range was 0 – 296 kcal/day. The mean EEE for athlete and non-athlete groups in the current study, fall within similarly defined groups previously reported.

Independently measuring EEE or EI provides little in determining the energy status and health of the athlete. When used to estimate EA, more inferences can be made regarding energy status. It is critical the methods used to determine these variables are meaningful and accurate to result in EA values that are also meaningful.

Oscillating EA in the Athletes' Training Program

In the present study, it was observed that the athletes' individual daily EEE was highly variable across the 7-day recording period. Most athletes were studied during a time that was representative of their normal training program. For example, Athlete 5 had a 7-day EEE value profile of: 356, 549, 0, 1373, 366, 1465, and 324 kcal/day across the week. Athlete 14 had an EEE profile of: 1277, 0, 1075, 257, 1138, 958, 0 kcal/day. In both examples heavy exercise days were followed by lighter expenditure days or rest days. The average EEE of the above examples are 633 (Athlete 5) and 672 (Athlete 14) kcal/day despite having some days of 0 kcal/day. With low volume or rest days placed between high expenditure days there may be a chance for the

athlete to compensate for days that are suboptimal in EI. Since low EA, not exercise stress, is what alters LH pulsatility and bone markers, EA variability across the recording period was examined. EA values for Athlete 5 were: 38, 40, 53, *19*, 36, *22*, *20* kcal/kg FFM/day. EA values for Athlete 14 were 16, 52, *18*, *40*, 29, *35*, and *55* kcal/kg FFM/day. The underlined values indicate suboptimal EA of < 30 kcal/kg FFM/day. For both athletes underlined values occur between days where EA is above the threshold of 30 kcal/kg FFM/day.[1] The mean EA value for Athlete 5 and 14 are 33 and 35 kcal/kg FFM/day, respectively. Figure 5.0 depicts this variability for Athlete 14 compared to the EA mean for the entire athlete group across week 1.

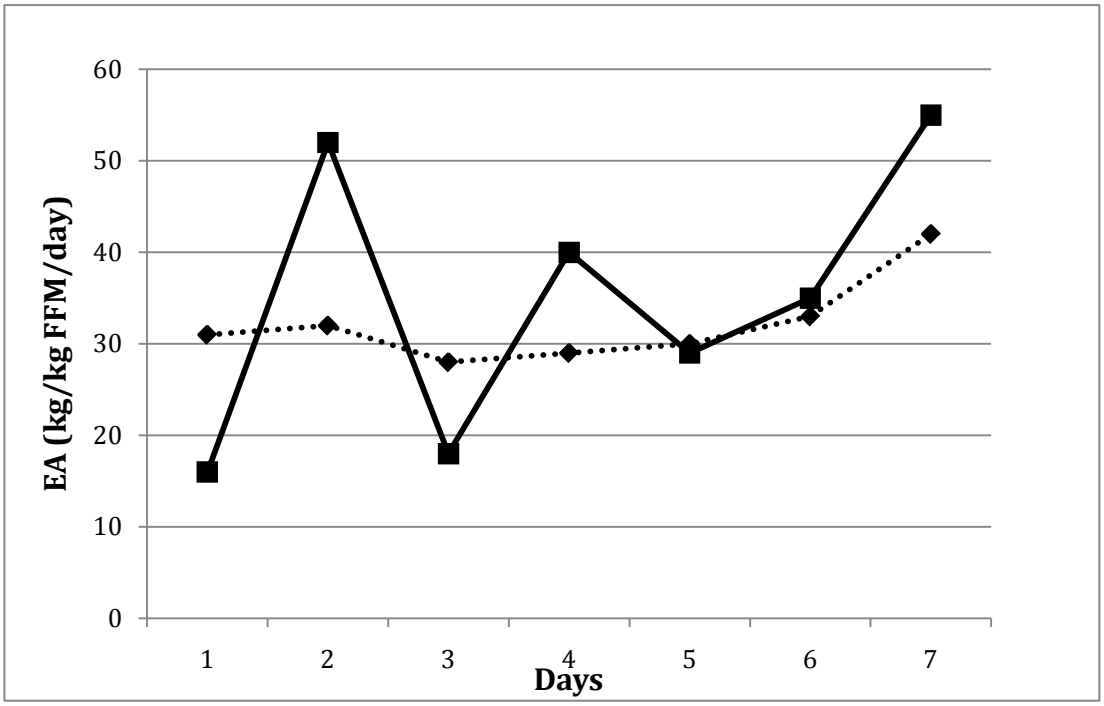

Figure 5.0. EA of an individual athlete (■, solid line) and the athlete group mean (♦, dashed line) across 7 days

Some athletes had negative EA values on a heavy training day followed by a substantially higher EA the next day. Much of an athlete's training cycle is periodized for load, intensity, and competition thus creating variability within EEE and therefore EA of the athlete. There were few athletes that had ≥ 5 consecutive days of low EA. Therefore, estimating EA over 1, 3, or even 5

days for an athlete may not accurately reflect her EA. In this study a sustained low EA across consecutive days was not observed hence alterations in LH pulsatility or bone markers are unlikely. The other studies measuring EA in athletes choose 3 day logs[20], 4 day logs[2], 2 sets of 3 day logs (where exercise value was taken for 7 days at baseline) [5], 7 consecutive days[6] and 7 days over 3 consecutive cycles[10]. Interestingly, the studies using the shortest time frame of 3 and 4 days, recorded the lowest EA values for the athletes.[2, 20]

Current EA recommendations are based upon a series of well-controlled studies completed on healthy sedentary females in a laboratory setting.[18,22,23] During those studies both the EI and EEE were controlled to create the EA for the subject groupings. The exercise treatment consisted of subjects jogging or running on a treadmill at a closely controlled setting where grade and speed were manipulated to ensure an intensity of 70% maximal oxygen consumption was met. Subjects needed to complete 15 kcal/kg LBM/day of this exercise protocol (results indicated this to be ~ 825 – 850 kcal/day for the subjects) during the 5 test days of the study.[18,23] Luteinizing hormone pulsatility characterizing menstrual function was disrupted at an EA threshold below 30 kcal/kg LBM/day. Similarly, Ihle et al, found the same relationship between EA and bone turnover using the same exercise protocol for subjects over 5 days of fixed exercise expenditure of 15 kcal/kg LBM/day.[19] The standardized high volume of daily EEE implemented in these controlled lab settings may not represent a typical weekly cycle of an athlete's training program as demonstrated in the current study. It may be most valuable to capture these data over a full periodization cycle of training for the athlete and not for short periods of time.

Low EA: Show me a sign! Female athletes taking OCP

The impact of low EA was investigated in sedentary, eumenorrheic women not taking OCP; low EA is linked to negative clinical consequences such as amenorrhea, impaired bone health, and cardiovascular dysfunction, its detection is of value. Since the menstrual cycle of female athletes taking OCP is suppressed, the ability to detect low EA and its associated consequences may be masked. Amenorrhea is an indicator that the energy needs of a female athlete may not be met. Reproductive function is an expendable process but still requires energy to function normally. During times of reduced energy intake, the essential processes will be prioritized (i.e. cell maintenance, circulation, neural activity) diverting energy away from the expendable processes (e.g. reproduction).[23] Therefore, the menstrual cycle becomes an indicator of energy status and its absence a red flag warranting attention. Because these reproductive markers were detected in response to low EA in women not taking OCP the outcome in the OCP population is unknown.

The whole body BMD was significantly higher (P=0.020) for the athlete group (1.18 ± 0.07) than the non-athlete group (1.12 ± 0.05). This result was expected as previous comparisons have found athletes typically having 5 – 15% greater BMD than control groups.[24] No athlete fell below a -1 Z-score for whole BMD. Specific site measurements (i.e. hip, lumbar, wrist) were not obtained. The results in these participants suggest that whole body BMD is within appropriate ranges and consequences of low EA such as BMD were not observed.

Although the participants of this study did not have BMD scores of concern reduced BMD is a well-documented consequence of exercise associated menstrual disturbances.[25] And OCP are often prescribed to 'treat' amenorrheic athletes as hormone therapy to further reduce or prevent BMD.[25] Only recently have studies begun to explore the potential consequences on bone health of premenstrual women already taking OCP. A systematic review by Lui and Lebrun,

found varying results when exploring the relationship of BMD and OCP across different categories of females.[25] Seven of 10 studies examining oligomenorrheic and hypothalamic amenorrheic premenopausal women taking OCP, revealed a positive effect on BMD. Lui and Lebrun found limited evidence of a positive effect of OCP on BMD in healthy premenopausal women; 29 of the 46 studies found no effect including all the randomized controlled trials.[25] Of greater concern is that 7 of the studies included in the review (cohort and cross sectional) suggested a negative effect of OCP on BMD in the healthy premenopausal women.[25] Therefore using BMD as a marker for low EA may be clouded by the independent negative effect OCP may have on bone health. Cobb et al, randomly assigned 150 competitive runners aged 18 – 26 years to either an OCP or control group for 2 years measuring BMD and BMC yearly. Their results were inconclusive with a trend towards protection for the oligo/amenorrheic group but for the eumenorrheic group no detrimental effects were noted.[26] Other studies have reviewed menstrual history as a specific variable associated with healthy bone formation. Hartard et al, studied the influence of OCP on BMD in young female endurance athletes, excluding oligomenorrheic and amenorrheic females.[27] In this retrospective analysis of 69 endurance athletes, they found that OCP use was negatively associated with the accrual of peak bone mass for the endurance athletes and that the age at which OCP use was initiated was a major determinant of spine BMD.[27] Reasoning that the OCP suppress endogenous sex steroid production and interfere with the rapid increase in skeletal mass during this critical time.[28] Hartard et al, completed a cross-sectional study on healthy females aged 18 – 24 years taking a low dose EE (< 50 ug) OCP formula. A major finding was that long duration and early start of OCP use was associated with a lower areal BMD of femoral neck, total bone mineral content at the distal tibia and tibial shaft.[28] And as with the earlier study by Hartard et al, gynecological age at OCP initiation was the best predictor of

total spine BMD.[28] For the athletes in the current study, the average time on the same OCP was 3.8 years with a range of 8 months – 13 years. On average, these athletes had been taking OCP for 35% of their gynecological age with one athlete taking the same OCP for >80% of her gynecological age. This value may be higher since many athletes mentioned that they had tried different OCP formulations in the past.

Review of the BMD component is outside of the scope of this study but perhaps some of the athletes may have not reached their peak bone formation due to OCP use at an early age. Without previous baseline measures of BMD, examining the impact of this issue is not possible. Using BMD as an indicator of low EA in those taking OCP may be confounded by the potential impairment of bone deposition during puberty. Additionally recent research studied the independent and combined effects of low estrogen (i.e. estrogen deficiency) and low energy (i.e. energy deficiency) on bone formation and resorption in exercising women.[28] When combined, these risk factors resulted in exacerbated bone turnover while in an adequate energy state regardless of estrogen status, bone formation and bone resorption was normal.[28] These findings emphasize the importance of energy status assessment of active women taking OCP. No studies to date have reviewed the effect of varying EA on bone markers or tracked BMD in female athletes taking OCP.

Additional indicators of low EA in female athletes are the disordered eating questionnaire scores. This study found no significant differences between the EDI and DEBQ-R scores between the groups. Although not diagnostic, the EDI and DEBQ-R can successfully discriminate between healthy and abnormal eating practices and behaviors and are validated to show differences between groups of athletes or sport disciplines. Previous studies have related disordered eating behaviors to exercise associated menstrual disturbances.[30] Particular subscales

of these tests, e.g. Drive for thinness, Body dissatisfaction, Cognitive Restraint, have been reported in studies to be higher in female athletes with cycle irregularity and/or eating disorders.[30,31] Use of these psychometric indices in female athletes taking OCP may help determine those that need support with their energy status.

Although not measured in this study, another sign of low EA that has been detected in female athletes not taking OCP is endothelial dysfunction. Endothelial function is measured by flow-mediated dilation and is an accepted marker of cardiovascular disease (the reader is referred to Zach et al, Hoch et al, and O'Donnell et al, for further reading).[32,33,34] Whether this is of concern for female athletes taking OCP with low EA remains unanswered. The effect of OCP on impaired endothelial function in amenorrheic athletes due to their hypoestrogenic has been examined.[35] Three groups (aerobically based amenorrheic athletes, regularly menstruating athletes, and sedentary controls) were given a low dose monophasic OCP and endothelial function was measured at baseline and after 9 months of treatment.[35] The OCP treatment significantly improved endothelial function in the amenorrheic athlete group, especially those with the lowest baseline values. The regularly menstruating athletes, who had the highest values at baseline, were unchanged.[35] Further studies are needed to explore the effects OCP may have on cardiovascular function in females with low EA.

Metabolic hormones and substrates (thyroid hormones, ghrelin, leptin, insulin, growth hormone, cortisol, glucose) effectively discriminate amenorrheic athletes from other menstrual categories identified in non-OCP users.[9] The effects of a low dose monophasic OCP on insulin, insulin-like growth factor binding protein 1, growth hormone, and cortisol were examined in three groups of age- and BMI-matched females (endurance athletes with menstrual disturbances, regularly cycling athletes and sedentary controls).[36] Following an 8-month treatment, hormone

levels were unchanged in regularly cycling athletes but were improved in the athletes with menstrual disturbances.[36] The effect of OCP may vary depending upon the baseline metabolic and hormonal state of the individual. The ability to detect low EA using these markers in athletes taking OCP requires further study.

Regardless if the female athlete is taking OCP, markers to indicate the health of female athletes is necessary. Determining an athlete's EI without discerning menstrual status, EEE, and possibly bone health has little relevance other than to comment that the value is high or low. More research is needed to distinguish whether the indicators of low EA in athletes taking OCP are similar to non-users. Individual monitoring becomes just as important for female athletes taking OCP because, as indicated above, other markers for non-OCP users may not be present. A combination of indicators is necessary to assess female athlete health and models for both OCP and non-OCP users are required.

Limitations of Study

The current findings contribute information regarding estimating EA in female athletes taking OCP. These findings do not explain the EA difference between the athlete and non-athlete group or the low EA value for the athlete group. A study comparing the EA of healthy athletes taking OCP and eumenorrheic menstruating athletes may reveal the impact of OCP on EA in these groups.

This study only reviewed OCP therefore inferences regarding other contraceptive methods such as intrauterine devices (IUD), the ring, patch, and extended pill cycle formulas cannot be assumed. Differences in pharmacokinetics due to the exogenous hormones being transdermally or paternally administered and cycle control (i.e. continuous cessation of menses for longer than 28 day cycle) were the main reasons for exclusion. However, future studies

should consider including additional contraceptive methods. Further, hormonal verification may help distinguish OCP 'dysfunctional' groupings or determine typical menstrual cycle profiles for female athletes taking OCP.

The use of a heterogeneous group of athletes may have produced a wider range of EA values. Research including homogeneous athlete groups (dancers) had far lower estimated EA.[2,20] However, significant EA differences between non-athletes and athletes were detected regardless of the sport therefore examining the energy status of athletes from different sport disciplines is recommended.

In this study EI, EEE, FFM, and REE were measured to estimate EA of the participants. As outlined in the methods a standardized protocol for each test was employed to reduce error for each measurement but equipment, technician, and reporting error may have led to inaccuracies in results. For example, using the MET to estimate EEE relies on the athlete to accurately describe exercise bouts in intensity and duration. Failure to do so limits the ability to assign an appropriate MET and can lead to under or over estimations in the EEE.[21]

EA, defined: (EI – EEE) / FFM = EA kcal /kg FFM/day, does not include post exercise oxidative consumption (EPOC) and non-exercise activity thermogenesis (NEAT). Considering the diversity of the athlete group the contribution of EPOC may alter the caloric amount for some athletes more than others. Although as a group the EA mean was far below the sedentary group, at an individual assessment level this value may explain higher dietary intakes for some of the sports versus others.[37] The EA definition also does not account for NEAT. The contribution of NEAT can account for more than daily EEE in some individuals.[38] Accounting for both NEAT and EPOC in the total energy expenditure for the athletes and the non-athletes may further lower their EA. For the sedentary participants their NEAT may contribute even more than the

purposeful exercise amounts recorded. This needs to be considered for future determination of EA in the practical setting.

Recommendations and Future Research

Research exploring female athlete health has seen a definite increase over the last few decades. Some dedicated research teams have established the foundation and set precedence in study design to help refute previous myths surrounding the energy status and menstrual function of female athletes. The contribution from animal experiments showing a relationship between reproduction and EA provided direction for study design in female athletes and the EA hypothesis. And since its conception, has grown to include many fields of research. Future target areas for EA are: continued research, education to female athletes and those involved in their development and health, and monitoring practices.

Research

Considering that half of female athletes use oral contraception, it seems unjustified to continue excluding 1 out of every 2 female athletes from benefiting from research that focuses on their health. Studies distinguishing the consequences of OCP use independent and in combination to that of low EA are future directions for research. Such a case of an amenorrheic OCP female athlete likely exists and the band-aid of exogenous hormones may only be a smoke screen of the consequences. OCP users may be included as a high-risk category for developing components of the female athlete triad seemingly at present they are flying under the radar. Determining the energy cost of a manipulated menstrual cycle and this impact on energy prescription compared to that of a normal menstrual cycle is needed.

Research estimating EA in athletes should carefully consider the duration of the recording period. As shown in this study accounting for both training and rest days across a week produces

highly variable EA. Therefore shorter recording times may not be reflective of the athlete's energy status.

As well, making an effort to incorporate newer technology for intake recording may reduce burden for the participants and possibly improve accuracy in the analysis. Numerous online dietary analysis websites already exist that are user friendly, readily available, and free. Hand held devices have dietary analysis applications that can be downloaded and assessed anytime. The access to nutrition labels has become readily available with companies and restaurants posting nutritional facts about products and menu items online. Online dietary programs often have a barcode scan option where scanning the food item results in the nutrition label being entered into the database. For the female athlete, using methods that are validated, cost effective, and convenient are the ideal combination.

Education

Collectively, research for the female athlete provides sufficient information to ensure better health for all female athletes. Female athletes need to understand and recognize how this information will impact their performance and health with emphasis early in the athlete's career. Recognizing the potential impact of early OCP use on bone health in young females plays a role in developing strategies and policies for the long term develop of female athletes. As the research suggests healthy bone formation occurs at critical times and is greatly affected by the components of the female athlete triad. Strategies and programs for delivery, prescription, and follow-up of the current findings from the research need to reach those that would benefit the most. Although further investigation surrounding low EA indicators and the female athlete taking OCP is needed, it should be addressed when educating this group.

Monitoring

Implementation of standard tracking and documentation of the female athlete's health throughout her career will not only help the athlete but aid in determining if education surrounding low EA and health is improving. It also provides a resource for research focus. Only one study to date has looked at the long-term implications of amenorrhea on bone health[8] and future studies are needed to understand the long-term effects on OCP use on bone, fertility, and metabolic substrates and hormones. Finding ways to ethically and conveniently implement monitoring methods for the benefit of female athletes may coincide with the education component.

Monitoring feedback may also include the sensitivity and usefulness of EA assessment tools. Research is often criticized for the lack of application to practical settings. Finding ways both groups can benefit (research and practical setting) is pivotal in creating meaningful results in this expanding field of interest.

The growing number of females participating in sport warrants research to continue distinguishing possible differences that occur due to gender and menstrual status variation to benefit both males and females. One EA prescription does not fit all; EA prescription should fit the athlete as opposed to the athlete fitting EA prescription. When prescribing an EA for the female athlete her stage of development, gynecological history, training status, psychological stress, and use of OCP should be considered.

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APPENDICES

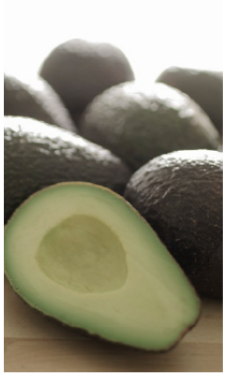
APPENDIX A

Recruitment Poster

UNIVERSITY OF ALBERTA

Faculty of Physical Education and Recreation

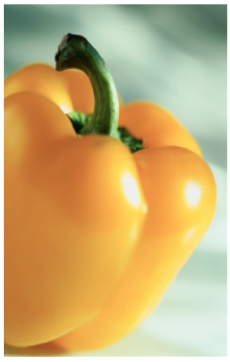
ENERGY AVAILABILTY IN FEMALE ATHLETES AND HEALTHY CONTROLS TAKING ORAL CONTRACEPTIVE PILLS



DO YOU WANT TO KNOW YOUR:

- Energy Balance
- Nutrient Analysis
- Body Composition
- Fitness Level
- Resting Metabolic Rate

If you are:



- ✓ A healthy female
- ✓ 18 – 35 years of age
- ✓ Using the same oral contraceptive pill for ≥1 year
- ✓ Stable body weight for the last 3 months
- ✓ Either
  - **ATHLETE** training > 10 hrs /week
  - **HEALTHY** female exercising ≤ 1 hour of aerobic exercise / week

Please contact either:

Kelly Drager: kdrager@ualberta.ca
Vicki Harber: Vicki.harber@ualberta.ca

PHONE: (780) 492 – 8739

Women's Health and Physical Activity Laboratory
Faculty of Physical Education and Recreation

APPENDIX B

Study Information Letter

UNIVERSITY OF ALBERTA

Faculty of Physical Education and Recreation

E488 Van Vliet Centre
Edmonton, Alberta, Canada T6G 2H9

STUDY INFORMATION LETTER

PROJECT TITLE: ENERGY AVAILABILTY IN FEMALE ATHLETES AND HEALTHY CONTROLS TAKING ORAL CONTRACEPTIVE PILLS

INVESTIGATORS:

Kelly Drager, RD, MSc Candidate *Phone: (780) 492-8739 (Kelly)*
Vicki Harber PhD (Professor) *Phone: (780) 492 -1023 (Dr. Harber)*

Women's Health and Physical Activity Lab E-455, Faculty of Physical Education and Recreation, University of Alberta

PURPOSE:

The purpose of this study is to compare the energy availability of female athletes and healthy females taking oral contraceptive pills. Energy availability is defined as dietary energy intake minus exercise energy expenditure.

BACKGROUND:

Irregular or absent menstrual cycles in active women is linked with lower bone mineral density, stress fractures and slower recovery from exhaustive exercise. Exercise by itself is not responsible for these events but seems to be due to low energy availability (defined above). Studies have shown that women with an EA below 30 calories/kg fat free mass (FFM)/day are most likely to experience the conditions described above. These studies have examined EA in sedentary women ONLY so we are not sure if this occurs in female athletes at a similar EA level. Also, the number of athletes taking oral contraceptive pills is growing. This group too has not been examined. We wish to measure EA in a group of healthy females taking OCP. This information will provide support for current nutritional recommendations and help maintain healthy menstrual function.

PROCEDURES:

We will study two groups of females taking oral contraceptive pills (OCP) across a two month period, assessing food intake, physical activity, and body composition (fat free mass; FFM) to estimate the EA of each person. One group will consist of inactive healthy females and the other group will be trained female athletes.

If you are eligible to participate in the study we will measure your height, weight, FFM, your eating attitudes, resting energy expenditure and aerobic fitness.

UNIVERSITY OF ALBERTA
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STUDY INFORMATION LETTER

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All of the above, except weight, will be measured once. Then you will complete two 7-day dietary intake and physical activity records; over 2 consecutive months, each record will start at the beginning of a new pill cycle; start date on day 1-4.

At your orientation meeting you will be asked to complete a 1-day food and activity record for practice and will receive feedback to improve accuracy and understanding of the task. You will be provided with a logbook for the dietary and exercise records.

TOTAL TIME COMMITMENT:

Orientation Meeting, Questionnaires and forms:	~ 1 hour
DXA Scan (body composition):	~ 30 min
Resting Energy Expenditure:	~ 1 hour
Aerobic Fitness (VO_{2Max}):	~ 1 hour
Total for Initial testing:	3.5 hours

2 sets of 7 day Dietary Intake and Physical Activity/Exercise Records:

15 – 30 min / day x 14 =	3.5 – 7 hours

Total time commitment per subject would be approximately 7 – 11 hours

RISKS:

There are no risks associated with answering the questionnaires or recording your dietary intake and exercise information. The resting energy expenditure procedure poses minimal to no risks to you. If at anytime during the procedure you wish to discontinue the test you may stop the testing.

The aerobic fitness test (VO_{2max}) may cause you some discomfort because you will be exercising to exhaustion. Injuries and adverse events that may result include muscle pulls, strains, cramps, dizziness, and feeling faint. You will be provided with instruction on performing the exercise test properly, reducing the risk of the above. All tests will be performed by qualified personnel who are trained to handle identifiable risks and emergencies, and have certification in CPR. The researchers will be watching for adverse symptoms at all times and will stop the test if they are concerned about your safety. You can also stop the test at any time. Please notify the researcher if during or after the test you experience any of the symptoms listed above.

Dual Energy X-Ray Absorptiometry (DXA) Scan will be performed to collect the necessary data on body composition. It is performed by a qualified technician. A separate information sheet and consent form for the DXA scan has been included for you to review and sign.

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STUDY INFORMATION LETTER

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BENEFITS:

The data from this study will help researchers further develop and understand the nutritional needs of exercising females to ensure both health and performance. As a participant you will be provided with reports of detailed personal fitness information, body composition, and nutrient analysis results. If you are interested in the research outcomes of this study, you may contact one of the researchers for this information.

CONFIDENTIALITY:

All information you provide and data collected will be confidential. All documents and files will be coded, and your name will not be attached to them. All documents will be stored in a locked room, and files on password protected computers and hard drives. When the study is presented or published, personal information that can be used to identify you will not be included. Research assistants completing data entry will comply with the University of Alberta Standards for the Protection of Human Research Participants and will sign Confidentiality agreement.

DATA STORAGE:

As the data files are coded, your name will not be attached to the files. All files will be stored on password protected computers and hard drives. Information is retained for a period of 5 years post publication, and when appropriate will be destroyed in a way that ensures privacy and confidentiality. The research will likely be presented at a research conference and published in a scientific journal.

FREEDOM TO WITHDRAW:

You can withdraw from the study at any time without consequence by simply informing one of the researchers. Your participation in this study is completely voluntary. If at any time you change your mind, you may withdraw from the study by verbally indicating your intent to the investigators. If you withdraw, your personal information will be removed from the study and any data that you have contributed to the study up to this point will be destroyed.

ADDITIONAL CONTACTS:

If you have concerns about this study you may contact Dr. Kelvin Jones, Chair of the PER-ALES Research Ethics Board, at 780-492-0650. Dr. Jones has no direct involvement with this project.

APPENDIX C

Consent Form

UNIVERSITY OF ALBERTA

Faculty of Physical Education and Recreation

E488 Van Vliet Centre
Edmonton, Alberta, Canada T6G 2H9

PARTICIPANT CONSENT FORM

PROJECT TITLE: ENERGY AVAILABILTY IN FEMALE ATHLETES AND UNTRAINED CONTROLS TAKING ORAL CONTRACEPTIVE PILLS

Do you understand you have been asked to be in a research study? YES NO

Have you read and received a copy of the study information sheet? YES NO

Have you been informed and understand the possible benefits and risks involved in taking part in this study? YES NO

Have you had the opportunity to ask questions and discuss the study with the researchers? YES NO

Do you understand that you are free to withdraw from this study at any time without prejudice, and that your information will be withdrawn at your request? YES NO

Has the issue of confidentiality been explained to you? Do you understand who will have access to you information? YES NO

Do you give permission for the use of the data collected from this study to be used in future studies (if this occurs, research ethics board approval would first be needed). YES NO

This study was explained to me by: _____

I agree to take part in this study:

_____ _____
Participant Name (print) Participant Signature and date

I believe that the person signing this form understands what is involved in the study and voluntarily agrees to participate.

_____ _____
Investigator Name (print) Investigator Signature and date

Questions or concerns contact Kelly Drager @ (403) 492 – 8739 or email: kdrager@ualberta.ca
or Dr. Vicki Harber @ (403) 492 – 1023 or email: Vicki.harber@ualberta.ca

APPENDIX D

Initial Questionnaire

Initial Questionnaire

To Be Completed By Subject **Date:**

Last Name: First Name:

Contact Information:

Local Phone: Cell Phone: Email:

Date of Birth (Day, Month, Year) Age Weight: Height:

FOR RESERACHER: BMI _____

Emergency Contact: Relationship:

Phone:

Any known medical conditions? (eg. Diabetes, polycystic ovarian syndrome (PCOS), thyroid disorders, cardiovascular disease, high blood pressure, depression) YES ☐ NO ☐
Which ones?:

Do you smoke? YES ☐ NO ☐

Other Information:

1. How old were you when you had your first period?
2. How many periods do you usually have in a year?
3. How long do your periods last?
4. When was your last period?
5. If you are not currently having a menstrual cycle, when was the last time you menstruated?
6. Do you ever have trouble with heavy bleeding? YES ☐ NO ☐
7. Do you ever experience cramps during your period? YES ☐ NO ☐
    a. If so, how do you treat them
8. What brand of oral contraceptive pill are you currently taking?
    a. How long have you been taking it?
9. Have you ever given birth? YES ☐ NO ☐
10. Have you ever been on a diet to reduce body weight? YES ☐ NO☐
    a. If yes, how many times have you tried to lose weight?
11. Have you ever tried to lose weight by: YES NO
    a. By vomiting? ☐ ☐
    b. By using laxative pills? ☐ ☐
    c. By using diuretics? ☐ ☐
    d. By using diet pills? ☐ ☐
12. Have you ever been diagnosed with an eating disorder? YES ☐ NO ☐
    (Example: anorexia nervosa or bulimia nervosa?)

13. Have you ever had a stress fracture? YES ☐ NO ☐
    a. If so, what bone has been injured?
        i. Indicate month/year:
14. Have you ever had a bone mineral density scan with a DEXA machine? YES ☐ NO ☐
    a. If so, indicate month/year:

Physical Activity:

	YES	NO
1. Are you involved in a regular routine of activity?.....	☐	☐

IF **NO**

Does your activity amount EXCEED 1 hour a week?.....	☐	☐

IF **YES** go to **Question 2**

2. Does your training include 3 or more sessions a week and / or > 10 hours / week?.....	☐	☐
a) How long have you been doing this routine for?		
3. Do you belong to a sport team / club or individual sport association?.....	☐	☐

IF YES:

Name of club/team:
Name of Sport:
Number of years competing:
What is the highest level of competition you have been involved in?
i.e. a) intramurals b) city c) provincials d) varsity e) national f) international

4. Please list and describe all the physical activities you are involved with

Activity	Duration	Frequency (Sessions / week)	Intensity
Example: Running	60 minutes	3	**3**

***Intensity: 1 – not vigorous at all (very light) 2 – Somewhat vigorous (light) 3 – Moderately vigorous (medium)
4 – Vigorous (heavy) 5 – Extremely vigorous (very heavy)

APPENDIX E

Energy Intake Log and Instructions

FOOD INTAKE Booklet

Name: _____

Telephone: _____

Email: _____

Recording dates: (_____)

Take this booklet with you!

Food Recording Tips

Maintain your **USUAL EATING HABITS**. Record **EVERYTHING** that you eat and drink. Be as **ACCURATE** as possible when you do so!! Use measuring cups/spoons or weigh scales if possible. If in doubt about what to record, write everything down or save the information (i.e. food label) in case you need to use it later. Below are some tips about improving accuracy when recording your food intake.

Accurate Measurement

a) Read the weights or volumes of foods or drinks from packages or wrappers. Example: 1 milk carton = 250 ml, 1 juice box, chocolate bar, record the gram amount on the package, potato chips = record what the serving size is for the package and how many chips you had.
b) A "fistful" of meat = 100 gm, "fistful" veggies = 1 cup, 1 cheese single = 1 oz, handful = ¼ cup or 60 ml, thickness of palm = 3 oz or 90 gm.

Method of cooking Indicate how your food was cooked.

a) Example: fried, steamed, baked, broiled, BBQ, etc.
b) Record any additional oils or spices were used in the cooking process.
c) When cooking vegetables, record the state of the raw vegetable (canned, raw, frozen) and the method used to cook them.
d) take note of what you add to canned soups: water, milk or nothing.
e) Record what you add and the can size used.

"Extras" Don't forget the **EXTRAS**. Example: ketchup, mustard, mayonnaise, gravy, or butter.

Food types Be specific about **TYPES** of food/drink.

a) Dairy products can be tricky. Always record the % milk fat and take note of low fat items.
b) For breads, buns, submarine buns, please record if they are white or brown or any other kind.
c) Eggs come in 3 sizes; small, medium and large; record the one you eat.
d) Fruit juices are labelled "Drink", "Beverage" or "Real Fruit Juice:; provide this information. Also note if they are sweetened, unsweetened or from concentrate. Example: **cheddar** cheese, **2%** milk, margarine or butter.

**** Whenever possible, identify ***brand names of the foods***.

Cooked or Dry Measurement Indicate whether the food measurement was taken before or after it was cooked. Example: state whether you measured meats, rice or pasta **before** or **after** it was cooked. The energy content of 1 cup of uncooked rice is very different from 1 cup or cooked rice!!

Specific Parts Indicate the exact part of the food you ate or what was removed before eating. Example: chicken (white or dark, bone in or out, skin or skinless), baked potato (skin or skinless), grounded beef (lean, extra lean, or regular).

Labels Whenever possible, *attach* the nutritional information label from the container (box, can, bag) to your booklet. If you can't do this, write down the CHO, protein, fat and energy (kcal) per serving (include serving size).

Vitamins, Minerals or other Supplements Please record the vitamins, minerals and supplements you take. If you have a label or a website link to the actual product(s) please provide.

TEA AND COFFEE should be included as well along with the cream, milk and sugar you add.

Don't forget the fluids … **WATER, BEER, WINE, LIQUOR, SPORT DRINKS** etc.

Fast Foods Include FAST FOOD items by name. Example: McDonald's Pizza Hut, Wendy's.

Recipes Record the AMOUNT/VOLUME of ingredients, the number of servings or volume the entire recipe makes and how many servings of what volume you ate. Example: recipe makes 30 cookies and I ate 5, or recipe makes 10 servings of lasagne and I ate 2 servings or 1/5 of the lasagne. Please ***attach*** the written recipe (just ingredients and volume) in your booklet.

Restaurant Means When you eat at a restaurant (other than a fast food place), record the name of the meal you ate, list the different ingredients on your plate and quantities of each. If you had sauces or dressing, were they lightly or heavily covered. Many restaurants have nutrition facts online, so be specific about the food item and restaurant name.

DAY 1, Date: ____________________

TIME of DAY	FOOD / FLUD ITEM	AMOUNT	BRAND or ATTACH LABEL	METHOD OF PREPARATION
			DAY 1	

APPENDIX F

Exercise Log

NAME:

WEEKLY EXERCISE LOG

	EXERCISE	DURATION	INTENSITY (mph, HR, watts)	TIME OF DAY	BREAKS/ REST/ INTERVAL		**RESEARCHER ONLY**				
DATE:	Sunday					TOTAL	MET VALUE	Wt (kg)	Corr MET	kcal	Net kcal
	i.e. Running	60 minutes	7 mph	9:00 - 10:00 am	of 60 min, 10 min walking, at 4 mph	50	8				
DATE:	Monday					Totals	MET VALUE	Wt (kg)	Corr MET	kcal	Net kcal
DATE:	Tuesday					Totals	MET VALUE	Wt (kg)	Corr MET	kcal	Net kcal
DATE:	Wednesday					Totals	MET VALUE	Wt (kg)	Corr MET	kcal	Net kcal
DATE:	Thursday					Totals	MET VALUE	Wt (kg)	Corr MET	kcal	Net kcal
DATE:	Friday					Totals	MET VALUE	Wt (kg)	Corr MET	kcal	Net kcal
DATE:	Saturday					Totals	MET VALUE	Wt (kg)	Corr MET	kcal	Net kcal

APPENDIX G

REE Setup Protocol

UNIVERSITY OF ALBERTA

Faculty of Physical Education and Recreation

E488 Van Vliet Centre
Edmonton, Alberta, Canada T6G 2H9

	YES
Arrive minimum 30 minutes early for equipment set up a) Turn on ParvoMedics machine (power bar switch) at least 20 minutes prior test, pump/heater of machine needs to be on for at least 5 minutes) b) Get hood ready prior to subject arrival (Velcro sheet to hood) c) Set up cot and pillow before subject arrives, have blanket on hand to provide subject if needed d) Turn computer on e) Ensure correct gas tank is hooked up to machine (has yellow tag around neck and gases = 16.00 % O_2 and 0.998 % CO_2 f) Plug in flow meter (turbine unit goes on back of machine) ***do not turn on yet*** g) Flow Calibration – set up calibration tube and flow pump. 5 strokes, 1st <80, 2nd > 100, 3rd > 200, 4th, > 300, 5th > 400 Aim for consistent increments (i.e. if first 50, then aim for 150, 250, 350, 450. Avoid slamming pump arm into pump at end of stroke h) Gas Calibration -	
Software Set-up a) Utilities ➔ Procedure configuration b) Select gas sampling method to dilation c) Select Expiratory Temperature to Room Temperature d) Select Expiratory Humidity to be Room Humidity plus 20% remaining	
Patient Care a) Review subject has signed CONSENT FORM (just a check, should have been completed at initial meeting) b) Enter subject data into computer, measure weight and enter for that day c) Have subject lay on cot for ~ 20 minutes prior hood covering d) Place hood + drape over subject and tuck in the sides of the drape snugly to avoid any air leakage e) Connect breathing tube to the deflector port close to the mouth and to the flow pump f) **TURN** flow pump on as soon as subject is covered with hood	
Flow Pump Rate a) looking for this rate to stabilize somewhere between 15 – 30 L/min and diluted CO_2 is between 1.0 – 1.2% b) This is completed while subject is set up with hood – take ~ 5 – 10 minutes to stabilize	
START THE TEST a) ***At 5 minutes into test re-evaluate flow rate*** b) Continue the test for ~ 20 minutes c) REPORTS: print off necessary reports – Nutrition Report allows selection of start time for data analysis STOP THE TEST a) take of the hood from the subject	
Post TESTING a) clean mouth piece closest to mouth with Cidex b) wipe inside of the hood with wipe c) Change software settings back for maximal testing procedure unless doing another test	

Questions or concerns contact Kelly Drager @ (403) 492 – 8739 or email: kdrager@ualberta.ca or Dr. Vicki Harber email: Vicki.harber@ualberta.ca

APPENDIX H

EDI and DEBQ-R Questions

Eating Attitudes Questionnaire

This is a scale that measures a variety of attitudes, feelings, and behaviours. Some of these items relate to food and eating. Others ask you about your feelings about yourself. THERE ARE NO RIGHT OR WRONG ANSWERS SO TRY VERY HARD TO BE COMPLETELY HONEST IN YOUR ANSWERS. Read each question and mark the optical scoring sheet in the following manner:

a = always
b = usually
c = often
d = sometimes
e = rarely
f = never

Please do not mark your answers on these sheets!!

Be sure to fill in the complete circle on your form with the provided pencil.

Please answer each question very carefully. RESULTS ARE COMPLETELY CONFIDENTIAL. Thank you

Always	Usually	Often	Sometimes	Rarely	Never		
( )	( )	( )	( )	( )	( )	1.	I eat sweets and carbohydrates without feeling nervous.
( )	( )	( )	( )	( )	( )	2.	I think that my stomach is too big.
( )	( )	( )	( )	( )	( )	3.	I wish that I could return to the security of childhood.
( )	( )	( )	( )	( )	( )	4.	I eat when I am upset.
( )	( )	( )	( )	( )	( )	5.	I stuff myself with food.
( )	( )	( )	( )	( )	( )	6.	I wish that I could be younger.
( )	( )	( )	( )	( )	( )	7.	I think about dieting.
( )	( )	( )	( )	( )	( )	8.	I get frightened when my feelings are too strong.
( )	( )	( )	( )	( )	( )	9.	I think that my thighs are too large.
( )	( )	( )	( )	( )	( )	10.	I feel ineffective as a person.
( )	( )	( )	( )	( )	( )	11.	I feel extremely guilty after overeating.
( )	( )	( )	( )	( )	( )	12.	I think that my stomach is just the right size.
( )	( )	( )	( )	( )	( )	13.	Only outstanding performance is good enough in my family.

Always	Usually	Often	Sometimes	Rarely	Never		
( )	( )	( )	( )	( )	( )	14.	The happiest time in life is when you are a child.
( )	( )	( )	( )	( )	( )	15.	I am open about my feelings.
( )	( )	( )	( )	( )	( )	16.	I am terrified of gaining weight.
( )	( )	( )	( )	( )	( )	17.	I trust others.
( )	( )	( )	( )	( )	( )	18.	I feel alone in the world.
( )	( )	( )	( )	( )	( )	19.	I feel satisfied with the shape of my body.
( )	( )	( )	( )	( )	( )	20.	I feel generally in control of things in my life.
( )	( )	( )	( )	( )	( )	21.	I get confused about what emotion I am feeling.
( )	( )	( )	( )	( )	( )	22.	I would rather be an adult than a child.
( )	( )	( )	( )	( )	( )	23.	I can communicate with others easily.
( )	( )	( )	( )	( )	( )	24.	I wish I were someone else.
( )	( )	( )	( )	( )	( )	25.	I exaggerate or magnify the importance of weight.
( )	( )	( )	( )	( )	( )	26.	I can clearly identify what emotion I am feeling.
( )	( )	( )	( )	( )	( )	27.	I feel inadequate.
( )	( )	( )	( )	( )	( )	28.	I have gone on eating binges where I have felt that I could not stop.
( )	( )	( )	( )	( )	( )	29.	As a child, I tried very hard to avoid disappointing my parents and teachers.
( )	( )	( )	( )	( )	( )	30.	I have close relationships.
( )	( )	( )	( )	( )	( )	31.	I like the shape of my buttocks.
( )	( )	( )	( )	( )	( )	32.	I am preoccupied with the desire to be thinner.
( )	( )	( )	( )	( )	( )	33.	I don't know what's going on inside me.
( )	( )	( )	( )	( )	( )	34.	I have trouble expressing my emotions to others.

Always	Usually	Often	Sometimes	Rarely	Never		
( )	( )	( )	( )	( )	( )	35.	The demands of adulthood are too great.
( )	( )	( )	( )	( )	( )	36.	I hate being less than best at things.
( )	( )	( )	( )	( )	( )	37.	I feel secure about myself.
( )	( )	( )	( )	( )	( )	38.	I think about bingeing (overeating).
( )	( )	( )	( )	( )	( )	39.	I feel happy that I am not a child anymore.
( )	( )	( )	( )	( )	( )	40.	I get confused as to whether or not I am hungry.
( )	( )	( )	( )	( )	( )	41.	I have a low opinion of myself.
( )	( )	( )	( )	( )	( )	42.	I feel that I can achieve my standards.
( )	( )	( )	( )	( )	( )	43.	My parents have expected excellence of me.
( )	( )	( )	( )	( )	( )	44.	I worry that my feelings will get out of control.
( )	( )	( )	( )	( )	( )	45.	I think my hips are too big.
( )	( )	( )	( )	( )	( )	46.	I eat moderately in front of others and stuff myself when they're gone.
( )	( )	( )	( )	( )	( )	47.	I feel bloated after eating a small meal.
( )	( )	( )	( )	( )	( )	48.	I feel that people are happiest when they are children.
( )	( )	( )	( )	( )	( )	49.	If I gain a pound, I worry that I will keep gaining.
( )	( )	( )	( )	( )	( )	50.	I feel that I am a worthwhile person.
( )	( )	( )	( )	( )	( )	51.	When I am upset, I don't know if I am sad, frightened or angry.
( )	( )	( )	( )	( )	( )	52.	I feel that I must do things perfectly or not do them at all.
( )	( )	( )	( )	( )	( )	53.	I have the thought of trying to vomit in order to lose weight.
( )	( )	( )	( )	( )	( )	54.	I need to keep people at a certain distance (feel uncomfortable if someone tries to get too close).
( )	( )	( )	( )	( )	( )	55.	I think that my thighs are just the right size.

Always	Usually	Often	Sometimes	Rarely	Never		
( )	( )	( )	( )	( )	( )	56.	I feel empty inside (emotionally).
( )	( )	( )	( )	( )	( )	57.	I can talk about personal thoughts or feelings.
( )	( )	( )	( )	( )	( )	58.	The best years of your life are when you become an adult.
( )	( )	( )	( )	( )	( )	59.	I think by buttocks are too large.
( )	( )	( )	( )	( )	( )	60.	I have feelings I can't quite identify.
( )	( )	( )	( )	( )	( )	61.	I eat or drink in secrecy.
( )	( )	( )	( )	( )	( )	62.	I think that my hips are just the right size.
( )	( )	( )	( )	( )	( )	63.	I have extremely high goals.
( )	( )	( )	( )	( )	( )	64.	When I am upset, I worry that I will start eating.

For the remaining questions (#65 – #74), mark the optical scoring sheet in the following manner

a = very often
b = often
c = sometimes
d = seldom
e = never

NOTE: When responding to questions 65 and 70 ONLY you may choose "not relevant" (f) as your response

Very Often	Often	Sometimes	Seldom	Never			
( )	( )	( )	( )	( )	Not Relevant ( )	65.	"When you have put on weight, do you eat less than you usually do?
( )	( )	( )	( )	( )		66.	Do you try to eat less at meal times than you would like to eat?
( )	( )	( )	( )	( )		67.	How often do you refuse food or drink offered because you are concerned about your weight?

Very Often	Often	Sometimes	Seldom	Never			
( )	( )	( )	( )	( )		68.	Do you watch exactly what you eat?
( )	( )	( )	( )	( )		69.	Do you deliberately eat foods that are slimming?
( )	( )	( )	( )	( )		70.	*When you have eaten too much, do you eat less than usual the following day?
					Not Relevant ( )		
( )	( )	( )	( )	( )		71.	Do you deliberately eat less in order not to become heavier?
( )	( )	( )	( )	( )		72.	How often do you try not to eat between meals because you are watching your weight?
( )	( )	( )	( )	,		73.	How often in the evening do you try not to eat because you are watching your weight?
( )	( )	)	( )	( )		74.	Do you take into account your weight with what you eat?

APPENDIX I

Ethinyl Estradiol Metabolism

Human 17α Ethinyl Estradiol Metabolism

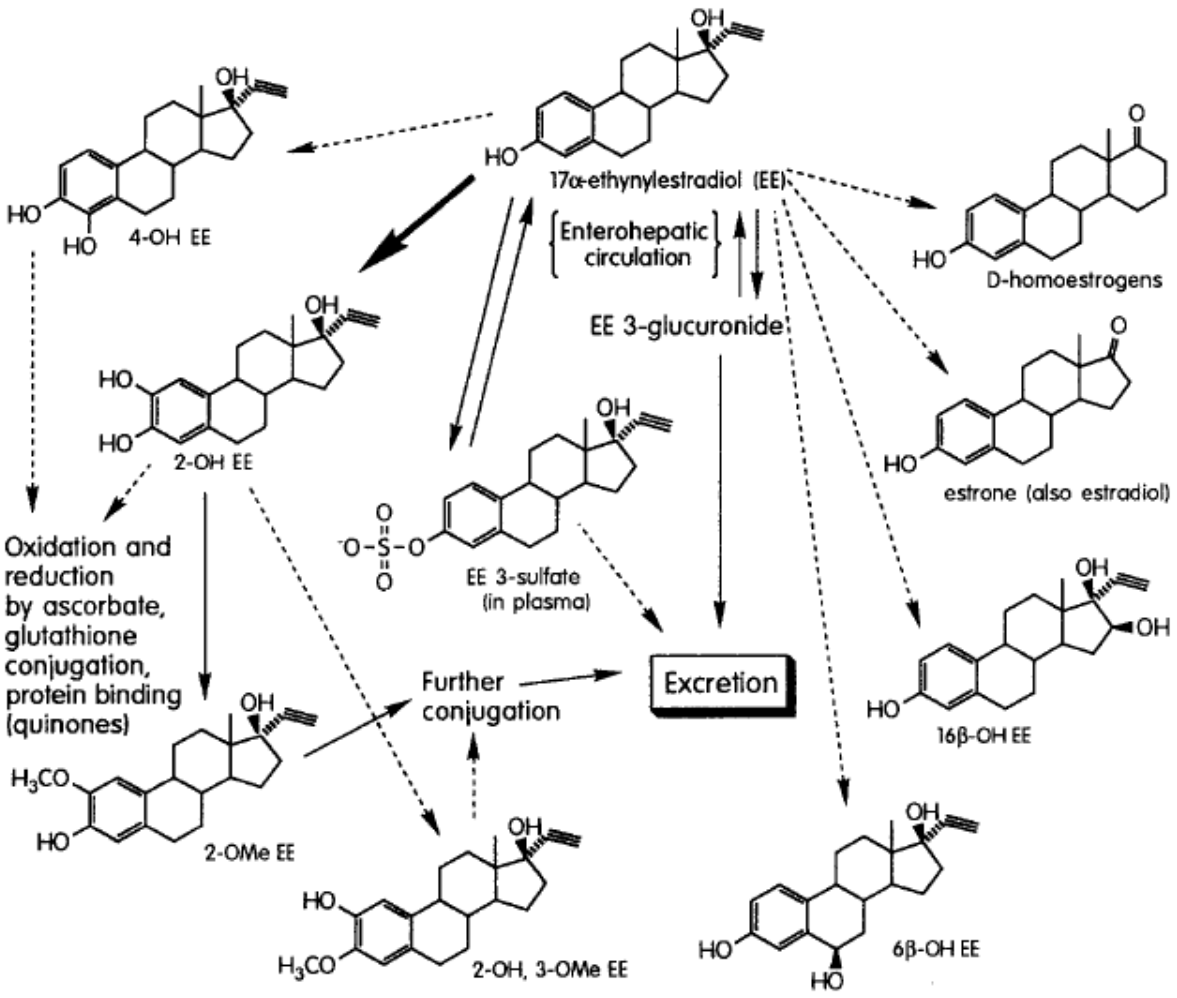


FIG. 1

Scheme of pathways involved in human 17α-ethynylestradiol (EE) metabolism, localized primarily in the liver. The more prominent pathways are indicated with heavier arrows. (Adapted from reference 7).

Source: Guengerich FP. MiniReview: Metabolism of 17α ethinylestradiol in humans. Life Sciences 1990; 47: 1981 – 1988.

APPENDIX J

EEE Calculations

Examples of 2 athletes and 2 non-athletes

Subject 1	Weight (kg)	REE 1480	Time min	MET Value	Duration hour	8-Apr	10-Apr	11-Apr	7-May	9-May	11-May
Light Bouldering	59	1.00	40	3.8	0.67	149.47					
High Wall	59		20	7.5	0.33	147.50					
Light Bouldering	59		20	3.8	0.33		74.73				
Cardio	59		30	8.3	0.50		244.85				
Stairs	59		20	15	0.33		295.00				
Problems	59		30	8	0.50		236.00				
5 x 5	59		30	8	0.50		236.00				
Light Bouldering	59		20	3.8	0.33			74.73			
Cardio	59		30	8.3	0.50			244.85			
5 x 5	59		20	8	0.33			157.33			
Problems	59		45	8	0.75			354.00			
Total Time			305								
Jogging 6.2mph	59		25	9.8	0.42				240.92		
Walking	59		5	6	0.08				29.50		
Conditioning	59		45	8	0.75				354.00		
(pullup, pushup	59										
abs)	59										
Light Bouldering	59		15	3.8	0.25				56.05		
Problems	59		90	8	1.50				708.00		
Light Bouldering	59		45	3.8	0.75					168.15	
5 x 5	59		25	8	0.42					196.67	
Problems	59		60	8	1.00					472.00	
Conditioning	59		10	8	0.17					78.67	
Light Bouldering	59		20	3.8	0.33						74.73
Problems	59		60	8	1.00						472.00
Conditioning	59		50	8	0.83						393.33
Total Time			450								
TOTAL						296.97	1086.58	830.92	1388.47	915.48	940.07

Cell C1: Calcululated correction factor

3.5/((REE/1440/5/body weight)*1000)

3.5 ml/kg/min = current value used for resting VO2

REE = measured REE of subjects

1440 = minutes in the day

5 = amount of kcal/L of oxygen

Body weight = weight in kg of subject

* 1000 = convert L to ml

WEEK 1 T	2214.47	kcal
CORR 1 T	2214.47	kcal
WEEK 2 T	3244.02	kcal
CORR 2 T	3244.02	kcal

Subject 6	Weight (kg)	REE 1847	Time min	MET Value	Duration hour	11-Apr	12-Apr	13-Apr	15-Apr	16-Apr	8-May	9-May	10-May	11-May	12-May	13-May	14-May
Bike	57.2	0.78	10	6	0.17	57.2											
Weight Lifting	57.2	0.78	60	10	1.00	572.0											
	57.2	0.78	42	11.8	0.70	472.5											
Stair Sprints	57.2	0.78	20	19.8	0.33		377.5										
Jog	57.2	0.78	40	11	0.67		419.5										
Bike Pyramid	57.2	0.78	35	14	0.58			467.1									
Weight Lifting	57.2	0.78	60	6	1.00			343.2									
Core	57.2	0.78	2	3.5	0.03			6.7									
Warm Up	57.2	0.78	10	6	0.17				57.2								
Speed Drills	57.2	0.78	3	23	0.05				65.8								
Speed Drills	57.2	0.78	9	2.8	0.15				24.0								
Weight Lifting	57.2	0.78	120	6	2.00				686.4								
Moksha Yoga	57.2	0.78	90	3.3	1.50					283.1							
Calisthetics	57.2	0.78	5	10	0.08						47.7						
Field Work	57.2	0.78	300	7.8	5.00							2230.8					
Yoga	57.2	0.78	90	5	1.50							429.0					
Field Work	57.2	0.78	300	7.8	5.00								2230.8				
Hurdles	57.2	0.78	30	10	0.50								286.0				
Cool Down	57.2	0.78	20	8.3	0.33								158.3				
Circus Training	57.2	0.78	5	10	0.08									47.7			
High Jump	57.2	0.78	60	6	1.00									343.2			
Shot Put	57.2	0.78	60	4	1.00									228.8			
Field Work	57.2	0.78	240	7.8	4.00										1784.6		
Gymnastics	57.2	0.78	120	4	2.00										457.6		
Moksha Yoga	57.2	0.78	75	3.3	1.25											236.0	
Heptathalon	57.2	0.78	23	10	0.38												219.3
Heptathalon	57.2	0.78	2	4	0.03												7.6
Warm Up	57.2	0.78	10	7	0.17												66.7
						44.6	294.5	364.4	44.6	220.8	37.2	1740.0	1740.0	37.2	1392.0	184.0	171.0
						446.2	327.2	267.7	51.3			334.6	223.1	267.7	356.9		5.9
Total week 1			501			368.5		5.2	18.7				123.4	178.5			52.1
Total week 2			1340						535.4								
TOTAL						1101.7	797.0	817.0	833.4	283.1	47.7	2659.8	2675.1	619.7	2242.2	236.0	293.6
						859.3	621.6	637.3	650.1	220.8	37.2	2074.6	2086.5	483.3	1748.9	184.0	229.0

Cell C2: Calcululated correction factor

3.5/((REE/1440/5/body weight)*1000)
3.5 ml/kg/min = current value used for resting VO2
REE = measured REE of subjects
1440 = minutes in the day
5 = amount of kcal/L of oxygen
Body weight = weight in kg of subject
* 1000 = convert L to ml

WEEK 1	3832	kcal
CORR 1	2989	kcal
WEEK 2	8774	kcal
CORR 2	6844	kcal

Subject 7	Weight (kg)	RMR 1510	Time min	MET Value	Duration hour	8-Apr	9-Apr	11-Apr	12-Apr	13-Apr	5-May	6-May	11-May
Walking	64	1.07	40	3.5	0.67	149.33							
Walking	64	1.07	60	3.5	1.00		224.00						
Walking	64	1.07	40	3.5	0.67			149.33					
Biking	64	1.07	30	10	0.50				320.00				
Walking	64	1.07	40	3.5	0.67					149.33			
Running	64	1.07	30	8.3	0.50						265.60		
Walking	64	1.07	40	3.5	0.67							149.33	
Biking	64	1.07	17	4	0.28								72.53
Total week 1			210			159.50	239.68	159.79	342.40	159.79	284.19	159.79	77.61
Total week 2			87										
TOTAL						149.33	224.00	149.33	320.00	149.33	265.60	149.33	72.53
						159.50	239.68	159.79	342.40	159.79	284.19	159.79	77.61

Cell C2: Calculated correction factor

3.5/((REE/1440/5/body weight)*1000)

3.5 ml/kg/min = current value used for resting VO2

REE = measured REE of subjects

1440 = minutes in the day

5 = amount of kcal/L of oxygen

Body weight = weight in kg of subject

* 1000 = convert L to ml

WEEK1 T	992.00	kcal
CORR1 T	1061.153	kcal
WEEK2 T	487.47	kcal
CORR2 T	521.59	kcal

Subject 11	Weight (kg)	RMR 1507	Time min	MET Value	Duration hour	31-May	27-Jun	2-Jul
Vo2max	63	1.05	9	9.32 kcal/min	8:38 min	78.10		
Biking	63	1.05	20	8	0.33		168.00	
Running	63	1.05	10	8.3	0.17			87.15
Biking	63	1.05	20	8	0.33			168.00
Weight Lifting	63	1.05	30	3.5	0.50			110.25
						78.10	176.40	91.51
Total week 1			9					176.40
Total Week 2			80					115.76
TOTAL						78.10	168.00	365.40
						78.1	176.4	383.67

Cell C2: Calculated correction factor

3.5/((REE/1440/5/body weight)*1000)

3.5 ml/kg/min = current value used for resting VO2

REE = measured REE of subjects

1440 = minutes in the day

5 = amount of kcal/L of oxygen

Body weight = weight in kg of subject

* 1000 = convert L to ml

WEEK 1 T	78.1	kcal
CORR 1 T	78.1	kcal
WEEK 2 T	533.40	kcal
CORR 2 T	560.07	kcal

APPENDIX K

Demographic Summary,
EI, EEE and EA Values

Table 1.0 Demographic Summary of Participants

(shaded boxes = athletes)

Subject	Age (yr)	Age Menarche (yr)	GA* (yr)	Height (cm)	Weight (kg)	BMI* (kg/m^2)	Fat Mass (kg)	FFM* (kg)	Fat % (%)	BMD* (g/m^2)	VO_2max (ml/kg/min)	REE* (kcal/day)
1	27	11	16	175	59.0	19.3	7.707	51.89	12.90	1.203	42.60	1480
2	22	14	8	168	61.0	21.6	13.805	46.90	22.80	1.098	52.90	1790
3	23	16	7	170	67.4	23.3	12.853	54.25	19.20	1.290	49.00	1867
4	21	12.5	9	158	55.0	22.2	15.214	39.19	28.00	1.094	39.40	1359
5	21	16	5	178	68.6	21.6	10.921	57.98	15.80	1.213	45.40	1937
6	22	16	6	174	57.2	18.9	8.713	48.59	15.20	1.058	45.50	1847
7	23	12	11	165	64.0	23.5	19.979	42.22	32.10	1.127	37.30	1510
8	32	13.5	18	173	56.5	18.9	8.925	47.08	15.90	1.225	48.50	1447
9	27	12	15	155	50.5	21.0	15.623	33.58	31.70	1.110	42.60	1338
10	23	12.5	10	165	68.0	25.0	18.620	48.48	27.70	1.280	40.90	1808
11	30	14	16	173	63.0	21.1	21.772	40.83	34.80	1.138	37.10	1507
12	29	14	15	160	57.7	22.5	16.929	39.57	30.00	1.193	36.40	1356
13	27	13	14	152	52.9	22.9	14.266	38.33	27.10	1.183	47.00	1653
14	20	13	10	175	60.3	19.7	8.379	52.22	13.80	1.210	54.20	1741
15	22	12	10	160	64.8	25.3	25.897	38.40	40.30	1.063	39.50	1560
16	24	12	12	150	49.2	21.9	18.044	29.46	38.00	1.077	32.30	1224
17	23	14	6	170	69.8	24.2	21.135	46.77	31.10	1.129	45.60	1830
18	20	10	13	167	64.3	23.1	14.779	49.32	23.00	1.240	42.60	1676
19	22	12	10	173	72.6	24.3	22.510	49.09	31.40	1.120	51.00	1781
20	28	12	16	160	75.0	29.3	29.700	43.40	40.60	1.135	32.30	1880
21	20	16	4	168	60.0	21.3	17.610	42.29	29.40	1.158	40.70	1713
22	31	10	21	168	69.0	24.5	27.723	39.88	41.00	1.196	32.50	1608
23	21	13	8	173	57.7	19.3	14.678	42.22	25.80	1.059	42.60	1650

* GA = gynecological age, BMI = body mass index, FFM = fat free mass, BMD = bone mineral density, REE = resting energy expenditure

Table 1.1 Summary of EI values for Participants

(EI kcal/day, shaded boxes = athletes, values are presented for both weeks)

	Day						
Subject	1	2	3	4	5	6	7
1	2508	2478	2040	2604	1492	1676	1968
1	2488	2625	2190	2178	1558	1496	2275
2	2122	2257	2020	2006	2219	2329	1961
2	2631	1802	2257	2505	2482	2095	3026
3	1917	1635	1783	1993	2379	1785	2057
3	1781	1906	2696	2253	2048	2646	1726
4	2062	1383	1346	1816	1767	1747	2381
4	1595	2252	2360	1826	2126	1823	1803
5	2569	2883	3065	2475	2456	2714	1498
5	3064	2854	2747	2267	2374	2651	4135
6	2295	1841	2030	2321	2665	2105	1996
6	1652	2171	1678	1613	2208	2576	2508
7	2253	1984	2133	2128	2446	2480	3189
7	1860	2340	2123	1504	3665	1200	2048
8	1770	1623	1141	2039	1604	1657	1702
8	1814	1516	1365	1570	1415	1887	1596
9	1747	2284	1257	2400	1656	2506	1836
9	1526	1702	1802	1538	2203	2518	2126
10	2091	2703	2474	2122	2748	2822	1742
10	2186	1523	1364	1866	1548	2014	1766
11	1976	2229	2107	2052	3076	2082	3067
11	2356	2323	2128	1994	2198	1888	1944
12	1818	1978	2413	2026	2145	2306	1211
12		2462	1454	1771	1810	1311	1649
13	2414	2392	2835	2591	2481	1919	2127
13	1895	2534	2333	2069	2448	1184	1902
14	3242	2613	2755	2575	2999	2592	2923
14	2100	2705	2014	2353	2652	2789	2894
15	1515	1907	1366	2331	2116	1525	2445
15	1256	2145	2119	2582	1893	1048	1098
16	1102	1394	1076	997	1870	1587	2190
16	1896	1752	1564	1571	1538	1567	2801
17	1474	1303	1345	1718	1149	1930	1966
17	1597	1671	1432	1440	1564	1612	1309
18	2291	2468	1985	2911	2787	2117	1485
18	3080	3236	2615	2792	2898	3001	3011
19	1965	1973	2320	2368	2289	2193	1932
19	1443	1947	1063	1189	1751	1457	2123
20	3091	1395	1482	3052	2699	2645	2050
20	2816	2495	2276	1850	1711	1804	1249
21	1984	2145	1941	2386	2348	1274	1692
21	1638	1604	1656	1799	2287	2272	2364
22	2513	2077	1661	1337	1551	1897	1733
22	2127	1740	1143	2222	2128	1187	1296
23	1333	1580	1921	1646	1005	2087	2144
23	1985	1730	1406	1385	1659	1317	2006

Table 1.2 Summary of EEE values for Participants

(EEE kcal/day, shaded boxes = athletes, values are presented for both weeks)

	Day						
Subject	1	2	3	4	5	6	7
1	297	0	1087	831	0	0	0
1	0	1389	0	916	0	940	0
2	682	1027	514	1080	462	0	938
2	692	258	818	393	763	354	0
3	327	674	0	507	169	401	474
3	1130	128	482	920	450	399	0
4	0	83	83	0	133	0	0
4	133	0	70	0	0	0	0
5	356	549	0	1374	366	1465	324
5	140	0	962	51	916	0	733
6	0	859	622	637	0	650	221
6	37	2075	2087	483	1749	184	229
7	0	160	240	0	160	342	160
7	284	160	0	0	0	0	78
8	553	208	161	415	0	1030	664
8	864	299	188	460	94	831	302
9	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0
10	1846	775	82	715	715	775	1256
10	1256	581	483	1321	581	715	0
11	0	78	0	0	0	0	0
11	176	0	0	0	0	384	0
12	180	108	180	180	0	231	216
12	0	0	86	0	593	556	0
13	228	514	234	1123	150	514	707
13	100	614	100	421	900	100	664
14	836	0	1404	1198	0	718	1093
14	1277	0	1075	257	1138	958	0
15	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0
16	0	0	39	0	0	168	0
16	0	253	0	0	0	0	0
17	1610	211	1273	0	0	0	0
17	1067	205	637	402	0	201	0
18	373	333	0	187	0	374	0
18	0	748	748	748	0	748	748
19	1492	465	1496	1043	1517	931	996
19	679	1399	213	381	730	550	550
20	189	192	0	0	0	0	216
20	0	0	288	0	384	0	0
21	62	62	62	62	62	92	0
21	62	246	62	205	62	123	0
22	187	233	279	0	0	0	0
22	298	186	559	149	0	0	0
23	0	244	0	0	0	0	89
23	0	0	0	0	0	0	0

Table 1.3 Summary of EA values for Participants

(EA kg/kg FFM/day, shaded boxes = athletes, values are presented for both weeks)

	Day						
Subject	1	2	3	4	5	6	7
1	42.6	47.8	18.4	34.2	28.8	32.3	37.9
1	47.9	23.8	42.2	24.3	30.0	10.7	43.8
2	28.3	22.7	30.3	16.0	35.9	49.7	18.6
2	41.3	32.9	30.7	45.0	36.7	37.1	64.5
3	29.3	18.8	32.9	28.2	41.0	26.2	30.0
3	12.0	32.8	40.8	24.6	29.5	41.4	31.8
4	52.6	33.2	32.2	46.3	41.7	44.6	60.8
4	37.3	57.5	58.4	46.6	54.3	46.5	46.0
5	38.2	40.2	52.9	19.0	36.0	21.5	20.3
5	50.4	49.2	30.79	38.2	25.2	45.7	58.7
6	47.2	20.2	29.0	34.7	54.8	29.9	36.5
6	33.2	2.0	-8.4	23.3	9.5	49.2	46.9
7	53.4	43.2	44.8	50.4	54.1	50.6	71.7
7	37.3	51.6	50.3	35.6	86.8	28.4	46.7
8	25.8	30.1	20.8	34.5	34.1	13.3	22.0
8	20.1	25.9	25.0	23.6	28.7	22.4	27.5
9	52.0	68.0	37.4	71.5	49.3	74.6	54.7
9	45.4	50.7	53.7	45.8	65.6	75.0	63.3
10	5.1	39.8	49.3	29.0	41.9	42.2	10.0
10	19.1	19.4	18.2	11.2	19.9	26.8	36.4
11	48.4	52.7	51.6	50.3	75.3	51.0	75.1
11	53.4	56.9	52.1	48.8	53.8	36.8	47.6
12	41.4	47.3	56.4	46.6	54.2	52.4	25.1
12	57.7	62.2	34.6	44.8	30.8	19.1	41.7
13	57.0	49.0	67.9	38.3	60.8	36.7	37.1
13	46.8	50.1	58.3	43.0	40.4	28.3	32.3
14	46.1	50.0	25.9	26.4	57.4	35.9	35.0
14	15.8	51.8	18.0	40.1	29.0	35.1	55.4
15	39.5	49.7	35.6	60.7	55.1	39.7	63.7
15	32.7	55.9	55.2	67.2	49.3	27.3	28.6
16	37.4	47.3	35.2	33.8	63.5	48.2	74.3
16	64.4	50.9	53.1	53.3	52.2	53.2	95.1
17	-2.9	23.3	1.5	36.7	24.6	41.3	42.0
17	11.3	31.3	17.0	22.2	33.4	30.2	28.0
18	38.9	43.3	40.2	55.2	56.5	35.3	30.1
18	62.5	50.4	37.9	41.4	58.8	45.7	45.9
19	9.6	30.7	16.8	27.0	15.7	25.7	19.1
19	15.6	11.2	17.3	16.5	20.8	18.5	32.1
20	66.9	27.7	34.1	70.3	62.2	60.9	42.3
20	64.9	57.5	45.8	42.6	30.6	41.6	28.8
21	45.5	49.3	44.4	55.0	54.1	27.9	40.0
21	37.3	32.1	37.7	37.7	52.6	50.8	55.9
22	58.3	46.2	34.6	33.5	38.9	47.6	43.5
22	45.9	39.0	14.6	52.0	53.4	29.8	32.5
23	31.6	31.6	45.5	39.0	23.8	49.4	48.7
23	47.0	41.0	33.3	32.8	39.3	31.2	47.5

APPENDIX L

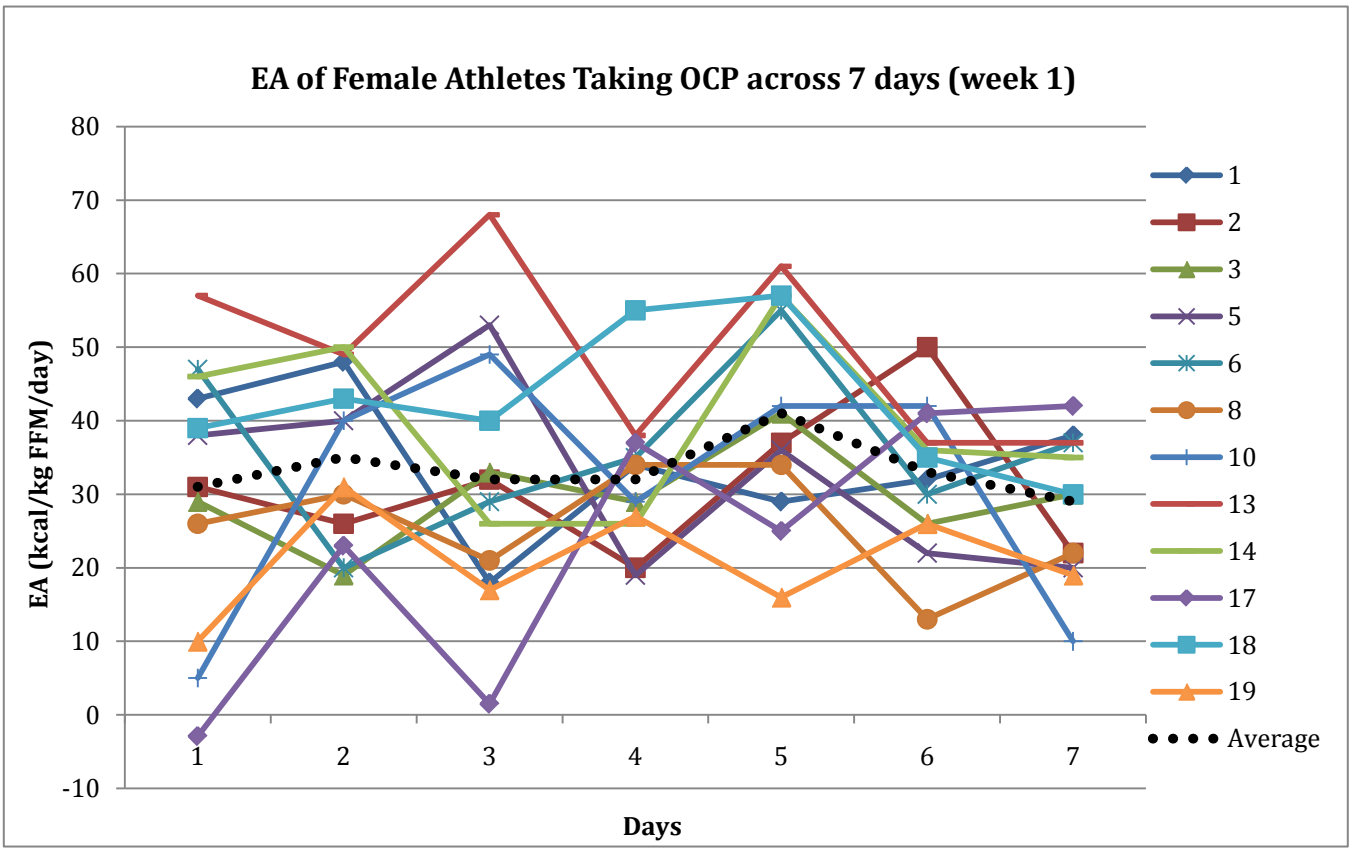
Statistical Results for EDI and DEBQ-R Correlations

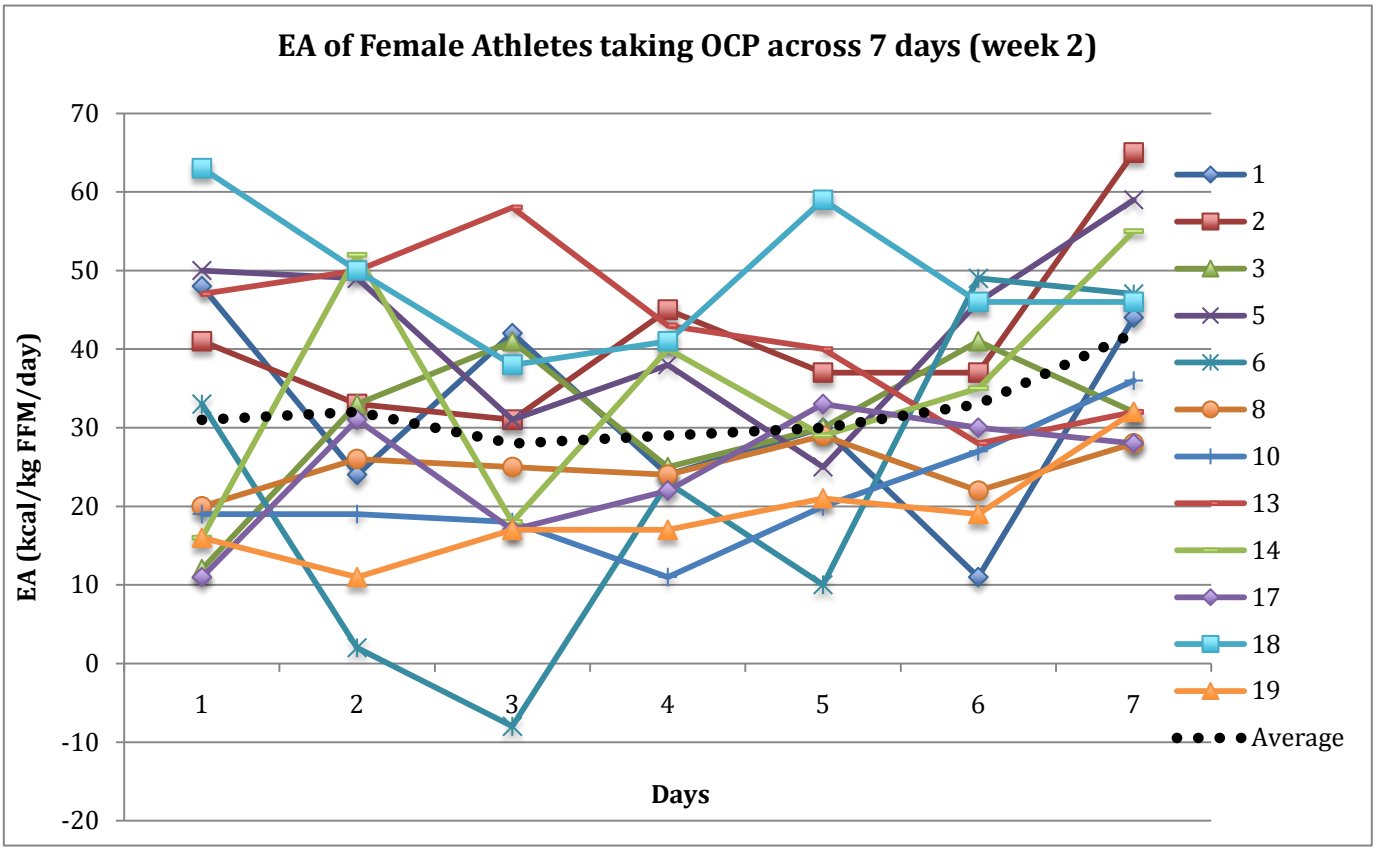
Relation between EDI subscale and DEBQ-R scores and Dietary Intake

Relationship with Dietary Intake	r value	r^2 value	P value
Drive For Thinness	0.056	0.003	0.801
Drive For Thinness WK2	-0.274	0.075	0.205
Bulimia	0.178	0.031	0.417
Bulimia WK2	-0.227	0.051	0.299
Body Dissatisfaction	-0.710	0.504	0.746
Body Dissatisfaction WK2	-0.523	0.274	0.010
Dietary Restraint	0.261	0.068	0.230
Dietary Restraint WK2	-0.014	<0.001	0.950

APPENDIX M

EA of Female Athletes taking OCP across 7 days


EA of Female Athletes Taking OCP across 7 days (week 1)
EA (kcal/kg FFM/day)
80
70
60
50
40
30
20
10
0
-10
1
2
3
4
5
6
7
Days
1
2
3
5
6
8
10
13
14
17
18
19
Average


EA of Female Athletes taking OCP across 7 days (week 2)
EA (kcal/kg FFM/day)
70
60
50
40
30
20
10
0
-10
-20
1
2
3
4
5
6
7
Days
1
2
3
5
6
8
10
13
14
17
18
19
Average