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The purpose of this project has been to determine the relationship between menstrual function, gender and total energy expenditure (TEE) on measures of bone health in males and females exposed to seasonal arduous work (extended operations). A secondary purpose of this project has been to determine the effect of gender and the menstrual cycle on substrate utilization (glucose metabolism) during exercise. Because the critical issues cannot be represented by a single investigation, this project involves a series of investigations that will be used to better determine some of the underlying health issues surrounding females in arduous work environments. The series of investigations that are being currently conducted will be referred to in the order in which they have been initiated. This includes, **I** - Effects of gender and the menstrual cycle on substrate utilization during exercise and **II** - Effects of menstrual function, gender, total energy expenditure (TEE), and the maintenance of energy balance on measures of bone health in males and females. At this time, data analyses and collection are still ongoing for Investigations **I** and **II**, respectively. Preliminary data findings are presented in the text for both components.

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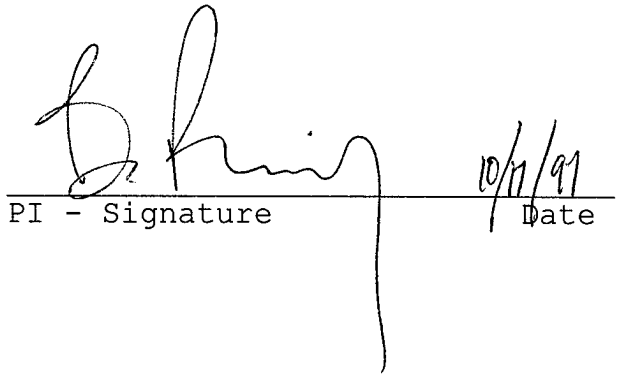

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Training and Extended Operations in Females: Effects on Reproductive Hormones, Bone Health, Task Specific Performance, and Comparative Energy Utilization

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I. Introduction

With the initiation of the women's marathon in the 1984 Olympic Games, the extent of female participants in endurance events has increased dramatically. Similarly, in the last decade, government agencies such as the USFS and US army have actively recruited females to serve as wildland firefighters and military recruits. Although the effects of regular exercise are thought to promote general health and well-being, intense exercise training in the female may actually compromise reproductive and bone health.

Compromised Menstrual Function and Bone Health

Regular intensive exercise training has been shown to diminish normal menstrual function in female participants (Boyden et al., 1983; Bullen et al., 1985; Shangold et al., 1979; Wilmore et al., 1992). If the exercise training persists, athletes can develop secondary amenorrhea (a cessation of the normal menstrual period) and unusually low plasma concentrations of circulating E2 (Boyden et al., 1983; Broocks et al., 1990; Schwartz et. al., 1981; Wilmore et al., 1992). This may contribute to abnormal bone health which has been demonstrated in even young athletes (Dhuper et. al., 1990; Hetland et. al., 1994). Similarly, Jones, et. al. (1993) reported that female army trainees had a significantly higher incidence of time-loss injuries compared to males (44.6 and 29.0% for females and males, respectively). Pester & Smith (1992) reported that the incidence in stress fractures was 0.91 and 1.09% in male and female soldier trainees, respectively. Whether a gender specific decrease in bone health contributes to the increased risk for injuries in the females remains unclear.

Although intensive exercise training can contribute to menstrual dysfunction, secondary amenorrhea does not always result. Boyden et al. (1983) progressively increased the weekly running mileage in a group of 19 healthy, regularly menstruating women by 30 and 50 miles per week above baseline. Although none of the women developed secondary amenorrhea, circulating levels of E2 decreased from 70.6 pg/ml at baseline to 33.6 pg/ml following the increase in training by 50 miles per week. However, the authors did not measure or mention the ramifications of decreased circulating E2 on bone health and risk of injury.

Hetland et. al. (1993) demonstrated that bone mineral content (measured via dual energy X-ray absorbtiometry) was negatively altered in only those females with amenorrhea. Although the assessment of reproductive hormone levels confirm menstrual disorders, it appears that significant bone loss (as detected by DEXA) is apparent only in females who develop amenorrhea.

Recently, Ruby et al. (1997) demonstrated that the use of biochemical markers (type I collagen cross-linked N-telopeptides) of bone turnover may provide a more dynamic, immediate trend in bone health in active premenopausal females compared to the traditional DEXA measure. In this study, 10 female cross-country runners were studied throughout their competitive season (approximately 12 weeks). Elevated bone resorption (BCE·μM creatinine^{-1}) was associated with subjects who demonstrated the following characteristics during the competitive season: **1)** progressive decrease in circulating estradiol and **2)** secondary amenorrhea.

Comparative Energy Expenditure: Gender Differences in Substrate Utilization

Results from past research have demonstrated at best a discrepancy in the existence and degree of substrate utilization between genders. In general, some studies have suggested that females have the capacity to rely more heavily on lipid sources while preserving muscle glycogen (Blatchford et. al., 1985; Froberg & Pederson, 1984; Tarnopolsky et. al., 1990). Of the research that has been done to examine the degree to which gender alters fuel selection patterns, Tarnopolsky et. al. (1990) has attempted to match subjects and has controlled for a variety of extraneous variables know to alter substrate utilization. If all of the data regarding gender differences in substrate utilization is closely examined, the average respiratory exchange ratio (RER) appears consistently lower in females (Ruby and Robergs, 1994). Although previous research has reported discrepancies in carbohydrate and lipid metabolism between males and females, the ramifications for job related safety and performance remain unclear.

Purpose

The purpose of this project has been to determine the relationship between menstrual function, gender and total energy expenditure (TEE) on measures of bone health in males and females exposed to seasonal prolonged arduous work (extended operations). A secondary purpose of this project has been to determine the effect of gender and the menstrual cycle on substrate utilization (glucose metabolism) during exercise.

Due to the complex nature of field operations and the physiology of various occupational demands, the proposed investigation has a multifaceted purpose.

Because the critical issues cannot be represented by a single investigation, this project involves a series of investigations that will be used to better determine some of the underlying health issues surrounding females in arduous work environments.

The series of investigations that are being currently conducted will be referred to in the order in which they have been initiated. This includes, **I** - Effects of gender and the menstrual cycle on substrate utilization during exercise and **II** - Effects of menstrual function, gender, total energy expenditure (TEE), and the maintenance of energy balance on measures of bone health in males and females.

III. Body

Experimental Methods

The experimental methods are provided in the order in which the investigations were initiated. That is, data collection occurred during the Fall 1996/Spring 1997 "off season" (referring to the fire season) and during the 1997 fire season. Although data collection and analyses for investigation I is nearly complete, investigation II has occurred during the fire season of 1997 and is still in progress. Post season data collection will not be complete until mid November, 1997. Although the data analyses for this investigation are currently ongoing, those analyses that have been completed will be included in the results and discussion.

I - Effects of gender and the menstrual cycle on substrate utilization during exercise

Initially, our methods for determining substrate utilization were limited to the measurement techniques of indirect calorimetry and the respiratory exchange

ratio (RER). However, because of recent advances made available to our laboratory, we were able to approach this research question using a glucose kinetics investigation. With the controlled collection of laboratory data to determine gender and menstrual specific differences in substrate utilization, we have been able to make the necessary adjustments for more appropriate field collection time points and techniques.

Due to the nature of the wildland firefighting occupation, crews and personnel are not finalized until early June. However, it was our goal to develop an understanding of how gender and the menstrual cycle may alter substrate utilization before we proceeded with field data collection during the fire season. Therefore, we studied well trained males and females that met or exceeded the fitness requirements associated with wildland firefighters (type I Hot Shot crews).

Objectives and Assumptions

It has been the specific aim of this component of the project to determine how gender and the menstrual cycle may alter patterns of fuel or substrate utilization during exercise. Although, it has been suggested that the source of energy expenditure (substrate utilization) may vary between genders (Tarnopolsky et. al., 1990), the effects of the normal menstrual cycle have shown limited (Dibrezzo et. al., 1988) to subtle (Higgs & Robertson, 1981) changes in work capacity. While males may work at a higher "self-selected" occupational intensity, they may also preferentially utilize more muscle and liver glycogen and therefore rely less on intramuscular and circulating lipid sources (triglycerides). If males and females differ in terms of substrate utilization, dietary demands during exercise and

extended occupational settings may vary between genders. Because it has also been suggested that females do not respond the same to carbohydrate loading or glycogen super compensation (Tarnopolsky et. al., 1995), a modified "combat ration" or "MRE" diet may be appropriate across genders. It has been the specific goal of this component to determine any discrepancy in substrate utilization and dietary demand relative to gender and the menstrual cycle so as to provide females with the most appropriate nutritional information.

Hypotheses and Expected Results

1. **There will be no difference in the percentage of the total oxidized carbohydrate and fat substrates during exercise between genders.**

Due to the heterogeneity of protocols, the matching procedures, and the general disregard for menstrual status, it is difficult to derive a directional hypothesis from prior research. Several past studies have concluded that females oxidize more fat relative to males (Friedmann & Kindermann, 1989; Froberg & Pedersen, 1984; Jansson et al., 1986; Tarnopolsky et al., 1990). Several other studies have concluded that there are no gender differences in the proportion of oxidized carbohydrates and fats during exercise (Powers et al., 1980; Wallace et al., 1980; Costill et al., 1979; Mendenhall et al., 1995). In all the reviewed studies on gender differences except one (Wallace et al., 1980), the sole criteria for exercise intensity was a set percentage of VO_2max. Subjects with the same VO_2max can have very different fuel selection patterns at similar exercise intensities if their lactate thresholds (%VO_2max) are different (Coggan et al., 1992). When Wallace et al. (1980) had active males and

females run at 89% of their respective lactate thresholds they discovered no differences in carbohydrate and fat oxidation.

In two studies where there were no gender differences in substrates oxidized, the male and females were matched on VO_2max expressed per kg body weight (Powers et al., 1980; Costill et al., 1979). Since females generally have less relative muscle mass (i.e., higher fat %), the females matched on VO_2max per kg body weight (ml/kg/min) may be expected to be more fit than the males. Although the subjects in the above studies may have had similar VO_2max and similar training this does not ensure similarities in lactate thresholds. Therefore, conclusions about gender differences from these above studies are tenuous because the differences could have simply been due to higher lactate thresholds in the females.

Since it has been shown fairly conclusively in rats that ovarian hormones can affect carbohydrate metabolism during exercise, the hormonal status of the female subjects should be taken into account in gender comparison studies. Only two reviewed studies (Tarnopolsky et al., 1990; Mendenhall et al., 1995) have controlled for this variable. Lavoie et al. (1987) and Hackney et al. (1994) concluded that the menstrual phase of the female plays a role in substrate selection patterns.

Since most of the reviewed studies did not control for the potentially confounding effects of matching subjects and exercise intensity with VO_2max, and menstrual status, a directional hypothesis about gender differences in substrate selection cannot be determined.

2. **There will be no difference in the percentage of the total oxidized carbohydrate and fat substrates during exercise between mid-luteal and mid-follicular phases of the menstrual cycle.**

Collectively, the majority of the literature suggests that there may be slight differences in metabolic responses to exercise between phases of the menstrual cycle. Only one reviewed study has determined a difference in fat and carbohydrate oxidation between menstrual phases (Hackney et al., 1994). Carbohydrate oxidation was higher in the follicular phase at exercise intensities of 35% and 65% of VO_2max. Bonen et al. (1983 & 1991), Jurkowski et al. (1981), Nicklas et al. (1989), and Kanaley et al. (1987) determined that there were no differences in fat or carbohydrate oxidation between menstrual phases. It has been shown in rats (Hatta et al., 1988; Kendrick et al., 1987; Rooney et al., 1993) that estradiol treatment can decrease carbohydrate oxidation and increase fat oxidation. Since circulating estradiol levels are increased in the luteal phase relative to the follicular phase, it may be expected that carbohydrate oxidation in the luteal phase would be decreased. This may be the case if estradiol acts alone on substrate selection or its action is not affected by other ovarian hormones that are in constant flux throughout the cycle. The ovarian hormone, progesterone mirrors the rise in circulating estradiol in the luteal phase and has been shown to suppress the effect of estradiol on carbohydrate metabolism (Hatta et al., 1988).

The majority of the reviewed literature suggest that fat and carbohydrate oxidation is similar between the follicular and luteal phases of the menstrual cycle.

3. **There will be a greater relative contribution of plasma derived glucose to total carbohydrate oxidation (Rd glucose/total carbohydrate oxidation) during exercise in females in the mid follicular phase of the menstrual cycle compared to males.**

There has only been one published study investigating the contribution of plasma derived and intramuscular glycogen to total carbohydrate oxidation (Mendenhall et al., 1995). In this study four males and four females in the follicular phase performed 60 minutes of cycle ergometry at 50% VO2peak. Free fatty acid and glucose stable isotope tracers were infused during exercise to estimate the contribution of intramuscular and extramuscular sources to substrate oxidation. The females used relatively more plasma derived glucose than the males. This is the only gender comparison study that has used stable isotope tracers to compare the relative contributions of intramuscular and extramuscular sources to substrate oxidation.

4. **There will be no difference in the relative contribution of plasma derived glucose to total carbohydrate oxidation (Rd glucose/total carbohydrate oxidation) during exercise in females in the mid luteal phase of the menstrual cycle compared to males.**

The relative contribution of plasma derived glucose to total carbohydrate oxidation between males and females exercised during the mid luteal phase has not been examined. Since females in the luteal phase have not been examined with the tracer or muscle biopsy technique in a gender comparison study, the direction of this hypothesis cannot be discerned.

5. **There will be no difference in the relative contribution of plasma derived glucose to total carbohydrate oxidation (Rd glucose/total carbohydrate oxidation) during exercise in females between the mid follicular and mid luteal phases of the menstrual cycle.**

No study has investigated menstrual phase effects on the relative contributions of hepatic and muscle stores to total carbohydrate oxidation during exercise. Only one study has examined the effects of menstrual phase on intramuscular glycogen utilization during exercise (Nicklas et al., 1989). This study demonstrated that there were no differences in intramuscular glycogen or total carbohydrate oxidation during exercise. If there were differences in the relative contribution of plasma derived and intramuscular glycogen during exercise, it would be expected that total carbohydrate or intramuscular glycogen would be different between phases. If one of these variables was different between phases and the other was not, then it would be expected that there are differences in relative contribution of plasma derived glucose to total carbohydrate oxidation.

Procedures

Setting

All physiological testing took place in the University of Montana's Department of Health Human Performance Laboratory, McGill Hall #121.

Subjects

A group of 5 males and 6 eumenorrheic females served as subjects for this investigation. The sample was comprised of recreational athletes aged 23-33 years. The subjects that volunteered to participate in this study completed a University of

Montana IRB-approved informed consent form. Height, weight, percent body fat, cycling VO_2 peak, and cycling lactate threshold were determined before completing one, two-staged submaximal cycling trial for the males and one, two-staged submaximal cycling trial during the mid follicular phase and one trial during the mid-luteal phase for the females. The two exercise intensities for the two-stage submaximal trials were set at 70% and 90% of the VO_2 at the lactate threshold.

Descriptive Data

Height, weight, age, menstrual status, and training habits

Data was collected to determine the participants height, weight, age, menstruation status, and training habits. All female subjects were asked to record their days of menses and their morning oral temperature for two months prior to all exercise trials on a provided calendar in order to accurately predict the time of mid-follicular and mid-luteal phases. None of the females were using oral birth control during the study.

Body composition

Body fat and lean body mass was assessed by hydrostatic weighing at residual lung volume. Residual lung volume was calculated using a Helium dilution technique. Percent body fat was calculated from body density using a Lohman (1992) age/gender specific equations.

Exercise testing

For all metabolic testing a TEEM 100 (Aerosport Inc., Ann Arbor, MI) metabolic system equipped with a medium-flow (10-120 l/min) pneumotach was used to analyze expired oxygen and carbon dioxide concentrations and volumes. Prior to

each cycle test, the metabolic system was gas calibrated with known concentrations of O_2 and CO_2, and flow rate was calibrated with a 3 l syringe before each exercise test according to the manufacturer (Aerosport Operators Manual, 1993). Heart rate was continuously monitored using a telemetry chest strap heart rate monitor for all testing (Polar, Port Washington, NY). All cycling tests took place using the subject's bicycle fixed to a Schwinn Velodyne ergometer for control of power output.

Peak VO_2 cycling test

Data were collected every 20 seconds for VO_2 (l/min and ml/kg/min) and RER. The warm-up period was approximately five minutes. The testing protocol began with three 4-minute steady-state stages of increasing power outputs (males - 100W, 175W, 250W; females - 75W, 125W, 175W). Immediately after the third stage, the power output was increased 25 Watts every minute until volitional exhaustion. The criteria for VO_2 peak was a plateau in VO_2 or a RER>1.1.

Lactate threshold test

In order to appropriately prescribe exercise intensities for the two-stage submaximal exercise trial, a lactate threshold test was performed. Before the test, an indwelling venous catheter was placed in an antecubital vein for blood sampling during exercise. The exercise protocol consisted of an initial workload (100 Watts for males, 50 Watts for females) for the first minute followed by an increase in power output of 25 Watts every minute until the females and males reached 100W and 200W, respectively. The power output was increased 15 W every minute thereafter until ~90%VO_2max. Blood (~1-2 ml) was sampled during the last 15 seconds of each 1-minute stage. Five hundred µl of blood was immediately deproteinized in 1 ml of

7% perchloric acid and frozen at -20∞ C until determination of blood lactate by an enzymatic assay (Lowry, 1971). Power output at lactate threshold was defined as the last workload before there was a curvilinear increase in plasma lactate concentrations. Three of the females performed this test in the luteal phase first and two performed the test in the follicular phase first. Menstrual phase has been shown not to affect the %VO2peak at which the anaerobic threshold occurs (Dombovy et al., 1987).

Two-stage submaximal exercise trial with 6,6- ^{2}H-glucose

Before the two-stage submaximal test, an indwelling Teflon catheter (18-20 gauge) was inserted into an antecubital vein of each arm. One catheter was used to prime the body and constantly infuse 6,6-^{2}H-glucose in 0.45% saline. A 60-ml syringe depressed by a Harvard infusion pump (Cambridge Isotopes Laboratories, Woburn, MA) was used to infuse approximately 3.0 µmol/kg/min 6,6-^{2}H-glucose for the first 10 minutes followed by 80 minutes of constant infusion at a rate of 0.42 µmol/kg/min. The catheter in the contralateral arm was used for blood sampling and was kept patent with a continuous saline drip. After the 80 minutes of constant infusion to obtain isotopic equilibrium, the subject cycled at a power output corresponding to ~70% of the VO_2 at their lactate threshold for 25 minutes immediately followed by 25 minutes of cycling at ~90% of the VO_2 at their lactate threshold. Blood samples were taken before priming (to assess background 6,6-^{2}H-glucose), 10 minutes, 5 minutes and immediately before exercise for resting glucose kinetics, and every 5 minutes during exercise for determination of 6,6-^{2}H-glucose,

glucose, lactate, and insulin concentrations (Figure 1). Expired gases were monitored during the last 5 minutes of each of the two stages for VO_2 and RER using the metabolic system mentioned above. To reduce the possibility that test familiarity would have an effect on the metabolic response, three of the five female subjects performed the two-stage submaximal test in mid-follicular phase first and the other two females performed the test during the mid-luteal phase first. Subjects were asked to refrain from eating 10 hours and exercising 36 hours prior to the two-stage submaximal exercise trial. All female subjects were instructed to eat a similar diet before each of their two trials and all subjects submitted a two-day dietary record before the two-stage submaximal exercise trials.

Figure 1. Blood sampling protocol for two-stage submaximal exercise trial (minutes)

priming dose | <----------constant infusion of 6,6-^{2}H-glucose----------> EXERCISE | -------70% LT------- | ------95%LT------>

3.0 µmol/kg·min 0.42 µmol/kg·min

-100	-90	-10	-5	0	5	10	15	20	25	30	35	40	45	50
G		G	G	G	G	G	G	G	G	G	G	G	G	G
				La				La	La				La	La
				Ins					Ins					Ins
			E2											

Abbreviations: G = glucose and 6,6-^{2}H-glucose; La = plasma lactate; Ins = insulin; E2 = estradiol

Metabolite and hormone assays

Glucose and lactate assays

Plasma from the necessary blood samples for glucose and lactate were obtained through centrifugation of the clotted blood samples and then frozen at -20°C. The supernatant was frozen at -20 °C until determination of blood glucose and lactate concentrations. Glucose and lactate concentrations from the two-stage submaximal trial were analyzed with a blood glucose and lactate analyzer (YSI, Yellow Springs, OH). All samples were analyzed in duplicate.

Hormones

Plasma from the necessary blood samples for hormone analysis were obtained through centrifugation of the clotted blood samples and then frozen at -20°C. Insulin (kit #TKIN2 - sensitivity of 1.5 mIU/mL) and estradiol (kit #KE2D1 - sensitivity of 1.4 pg/mL) were assayed for with commercially available double antibody radio-immuno assay kits (Diagnostic Products Corp., Los Angeles, CA). All samples were analyzed in duplicate.

Isotopic enrichment

The ratio of 6,6-^{2}H-glucose to unlabeled glucose (isotopic enrichment) was determined by forming a pentaacetate derivative of glucose and using a gas-chromatography mass spectroscopy technique as described by Wolfe (1992). The peak abundances of ions m/e 200 for glucose, m/e 201 and m/e 202 for 6,6-^{2}H-glucose were monitored for calculating the isotopic enrichment. A Hewlett-Packard 5980 Plus gas chromatograph-mass spectrometer with a packed column was used to

separate the pentaacetate derivative from other organic compounds. The pentaacetate derivative was fragmented with electron ionization of 70 keV and the ions of mass 200, 201, and 202 were selectively monitored for abundances to calculate the enrichment of the infused 6,6-^{2}H-glucose.

Calculation of glucose kinetics: Ra, Rd, glycogen and plasma glucose utilization

Glucose rates of appearance (Ra) and disposal (Rd) from the circulation were calculated with the Steele (1959) equation using non-steady state assumptions during the last 5 minutes of the two exercise intensities.

$$Ra=\frac{F-(VC)dE/dT}{E}$$

$$Rd=Ra-(V)dC/dT$$

where...

F rate of infusion of the isotope (umol/kg/min),

V effective volume of distribution for glucose (ml/kg),

C concentration of glucose (umol/ml),

E enrichment of the tracer (6,6-^{2}H-glucose : glucose ratio),

dE/dT instantaneous change in enrichment at a given time,

dC/dT instantaneous change in glucose concentration at a given time.

The time series smoothing technique of spline fitting was used to best approximate the change in isotopic enrichment (dE/dt) and change in glucose concentration (dC/dt) during exercise. The relative contribution of blood glucose to total carbohydrate oxidation was calculated using the average glucose Rd from the last 5

minutes of each exercise intensity as the rate of blood glucose oxidation. The oxidation of Rd glucose is between 96-100% at 50% VO2max (Jeukendrup et al., 1996). The difference between total carbohydrate oxidation and Rd determined the minimal rate of intramuscular glycogen oxidation. For the calculation of Ra, the effective volume of distribution for glucose was set at 100 ml/kg.

Research design and statistical procedures

The descriptive variables VO_2 max (ml/kg/min and ml/kg/min LBW) and $\%VO_2max$ at lactate threshold were analyzed with independent sample t-tests to compare males and females. Dependent sample t-tests were used to analyze differences between phases for single level factors (estradiol, body weight). All other dependent variables were analyzed based on a series of *a priori* planned comparisons (Shavelson, 1988). All planned comparisons were analyzed with SuperAnova statistical package (Abacus Inc, Berkeley, CA). The level of significance was set at an overall experimental alpha of 0.05.

*Outline of **a priori** planned comparisons*

1) Blood metabolites and hormones - comparison of follicular and luteal phases at rest, 20-25, and 45-50 minutes for the variables blood glucose, lactate, and insulin. (3 level, within factor) FF vs FL $p<0.033$. (3 level, between factor) M vs FF and M vs FL $p<0.017$.
2) Substrate selection - a) RER, % of total kcal from carbohydrate, and % of total kcal from fat using mean from time points 20 and 25 minutes of each exercise intensity. (2 level, within factor) FF vs. FL, $p<0.025$. (2 level, between factor) M vs FF and M vs FL $p<0.013$.

3) Source of oxidized carbohydrates - % of total oxidized carbohydrates from intramuscular glycogen and % of total oxidized carbohydrates from plasma derived glucose using the mean from time points 20 and 25 minutes of each intensity. (2 level, within factor) FF vs. FL, $p<0.025$. (2 level, between factor) M vs FF and M vs FL $p<0.013$.

Calculations for Type I error

1) FF vs. FL, (3 level, within factor) $p<0.033$ because the P(Type I error)=$1-(1-x)^{3/2}$ =0.05. x=0.033 for 3 comparisons (rest, low, high)
2) FF vs FL (2 level, within factor) $p<0.025$ because the P(Type I error)=$1-(1-x)^2$ =0.05. x=0.025 for 2 comparisons.
3) M vs FF and M vs FL (3 level, between factor) $p<0.017$ because the P(Type I error)=$1-(1-x)^{3/2}$=0.05. x=0.033 for 3 comparisons and since M compared twice (vs FF and FL) P(Type I error)=$1-(1-x)^2$ =0.033. x=0.017
4) M vs FF and M vs FL (2 level, between factor) $p<0.013$ because the P(Type I error)=$1-(1-x)^2$=0.05. x=0.025 for 2 comparisons and since M compared twice (vs FF and FL) P(Type I error)=$1-(1-x)^2$ =0.025. x=0.013.

Results and discussion

Subject characteristics

All of the subjects (n=11) of this study except one female (cyclist) were recreational triathletes who had participated in at least one triathlon in the last 12 months and were training for swimming, cycling, and running during the study. All expressions of cycle ergometer maximal oxygen consumption (l◊ min^{-1}, ml◊kg^{-1}◊ min^{-1}, ml◊kg^{-1} lean body mass ◊ min^{-1}) were significantly greater in the males ($p<0.01$). The males

were taller ($p<0.02$) and had a lower body fat percentage ($p<0.02$). While maximal heart rate and the % VO_2peak at the lactate threshold were lower in females these were not significant ($p=0.36$, $p=0.055$, respectively). All five females used in the male versus luteal phase female comparison were used in the menstrual phase comparison. One of the five females used in the male versus mid-follicular comparison was not used in the menstrual phase comparison because of a failed luteal phase trial. The physical characteristics of the 5 regularly menstruating females examined in the mid-follicular and mid-to-late luteal phases are presented in Table 1. Four of the five females used in the menstrual comparison were used in the gender comparison component of this study. All of the females were menstruating regularly for at least five months prior to the study and none of the females were using oral contraceptives during the study according to the responses of the menstrual history questionnaire.

Table 1. Physical characteristics of males (n=5) and regularly menstruating females (n=5). Expressed as mean ± standard error.

	MALES	FEMALES
Age (years)	25.0 ± 2.0	24.0 ± 1.0
Height (cm)	173.9 ± 1.8	165.3 ± 2.3*
Body Mass (kg)	68.0 ± 2.8	63.2 ± 3.4
Body Fat (%)	8.8 ± 1.4	16.4 ± 1.8*
Maximum Heart Rate ($b \cdot min^{-1}$)	191 ± 4.2	186 ± 2.5
VO_2peak ($l \cdot min^{-1}$)	4.20 ± 0.23	3.03 ± 0.14**
VO_2peak ($ml \cdot kg^{-1} \cdot min^{-1}$)	61.7 ± 1.3	48.6 ± 1.3**
VO_2peak ($ml \cdot kg^{-1}$ LBM $\cdot min^{-1}$)	67.4 ± 1.3	58.0 ± 1.2**
% VO_2peak @ lactate threshold	68.9 ± 2.6	62.1 ± 1.5

***p<0.05, **p<0.01**

Cyclical changes in serum estradiol were used to confirm the presence of a changing hormonal milieu consistent with a menstruating female. Anticipated rises in oral temperature were only confirmed in three of five subjects. Inadequate recording of morning oral temperature was most likely responsible for the lack of detection in the two females who discerned no increase in oral temperature, because both had elevated estradiol levels during the luteal phase.

Table 2. Physical characteristics of menstrual phase comparison subjects n=5. Expressed as mean ± standard error.

Age (years)	23.8± 1.1
Height (cm)	162.9 ± 3.0
Body Mass (kg)	59.3 ± 4.2
Body Fat (%)	15.2 ± 1.5
Maximum HR ($b \Diamond min^{-1}$)	184.4 ± 1.3
VO_2peak (l/min)	2.87 ± 0.21
VO_2peak ($ml \Diamond kg^{-1} \Diamond min^{-1}$)	48.9 ± 1.3
% VO_2peak @lactate threshold	60.1 ± 2.2
Age at menarche (years)	13.2 ± 0.2
Length of cycle (days)	28 ± 0.5

Expression of exercise intensity during the two-stage subthreshold trial

The planned intensity for the low intensity workload was 70% of the VO_2 at the lactate threshold (% VO_2 @ LT) and the high intensity was planned for 90% of the VO_2 at the lactate threshold. There were no differences in the % VO_2 @ LT, HR, and the %HRmax during the low intensity between the males and the follicular phase females nor between the males and the luteal phase females (Table 3). The luteal phase females tended to have lower heart rates than the males during the low intensity workload but this was not significant.

Since the lactate threshold of the males occurred at a higher percentage ($p=0.055$) of their VO_2 peak, the intensity when expressed as % VO_2peak was significantly higher for the males compared to both phases of the females during both low and high intensity workloads.

There were no significant differences in %HRmax between the males and both phases of the females, although during the high intensity workload, the luteal phase females tended to work at a lower %HRmax than the males ($p=0.019$). The follicular females worked at a significantly lower percent of their lactate threshold ($p=0.011$) than the males during the high intensity workload. Any deviation from the planned intensity was most likely due to insufficient prediction of the watts required to elicit the appropriate oxygen consumption and calibration error of the electronically braked cycle ergometer. During the high workload, the females in both phases had significantly lower heart rates than the males.

Table 3. Workload intensities for the two-stage subthreshold trial gender comparison. Expressed as mean ± standard error.

	Low Intensity			High Intensity		
Intensity	Male-Lo	Follic-Lo	Lut-Lo	Male-Hi	Follic-Hi	Lut-Hi
% VO_2 @ LT	68.7 ± 0.9	67.3 ± 2.7	69.5 ± 2.2	90.1 ± 1.1	83.9 ± 1.7*	88.9 ± 1.5
% VO_2peak	47.3 ± 1.5	41.8 ± 1.6*	41.7 ± 1.7*	62.2 ± 2.6	52.0 ± 1.1**	53.5 ± 2.3**
HR	129 ± 5.0	124 ± 5.9	119.9 ± 2.4	158 ± 6.8	149 ± 5.3*	142.7 ± 2.4*
%HRmax	67.5 ± 1.3	66.8 ± 3.1	65.0 ± 1.5	82.4 ± 2.5	80.0 ± 2.8	77.5 ± 1.2

Different from males *$p<0.013$ ($\alpha=0.05$), **$p<0.01$

There were no significant differences in oxygen consumption between the follicular and luteal phases at the low and high workloads (Table 4), although the

luteal high workload tended to be higher (p=0.07). However, heart rate was significantly lower at the higher workload during the luteal phase.

Table 4. Workload intensities for the two-stage subthreshold trial menstrual phase comparison. Expressed as mean ± standard error.

	Low Intensity		High Intensity	
Intensity	Follic-Lo	Lut-Lo	Follic-Hi	Lut-Hi
% VO_2 @ LT	69.8 ± 2.4	69.5 ± 2.2	85.3 ± 2.1	88.9 ± 1.5
% VO_2peak	41.8 ± 1.6	41.7 ± 1.7	51.1 ± 1.3	53.5 ± 2.3
HR	121.6 ± 4.7	119.9 ± 2.4	146.1 ± 4.4	142.7 ± 2.4**
%HRmax	66.0 ± 2.9	65.0 ± 1.5	79.3 ± 2.7	77.5 ± 1.2**

Different from follicular *p<0.025 (α=0.05), **p<0.01

Indirect calorimetry and substrate oxidation

According to respiratory exchange ratios (RER), there were no significant differences in the percentage of calories derived from carbohydrate and fat oxidation between the males and the females. However, the percentage of fat oxidized was greater in the luteal phase females than males and the percentage of calories derived from carbohydrate were lower, but this did not reach statistical significance (p=0.025 > 0.013 (α=0.05)).

Table 5. Carbohydrate and fat oxidation during the two-stage subthreshold trial gender comparison. Expressed as mean ± standard error.

	Low Intensity			High Intensity		
	Male-Lo	Follic-Lo	Lut-Lo	Male-Hi	Follic-Hi	Lut-Hi
RER	0.819 ± 0.010	0.826 ± 0.018	0.830 ± 0.021	0.851 ± 0.004	0.861 ± 0.018	0.837 ± 0.021
%CHO	40.5 ± 3.4	42.7 ± 6.4	44.1 ± 7.3	51.6 ± 1.5	55.4 ± 5.9	46.7 ± 6.9
%FAT	59.5 ± 3.4	57.3 ± 6.4	55.9 ± 7.3	48.4 ± 1.5	44.6 ± 5.9	53.3 ± 6.9

Different from males *p<0.013 (α=0.05), **p<0.01

Rates of carbohydrate and fat oxidation, and the percentage of calories derived from carbohydrate and fat at the lower workload were similar during the follicular and luteal phases (Table 6). At the higher workload, however, fat oxidation (g FAT/min) was increased significantly by 21% in the luteal phase compared to the follicular phase and the percentage of calories derived from fat (53.3% vs. 44.7%) were higher during the luteal phase. Correspondingly, carbohydrate oxidation (g CHO/min) was reduced by 10% during the high workload of the luteal phase and the percentage of calories derived from carbohydrates was lower in the luteal phase (46.7% vs 55.3%).

Table 6. Carbohydrate and fat oxidation during the two-stage subthreshold trial menstrual phase comparison. Expressed as mean ± standard error.

	Low Intensity		High Intensity	
	Follic-Lo	Lut-Lo	Follic-Hi	Lut-Hi
RER	0.831 ± 0.015	0.830 ± 0.021	0.863 ± 0.018	0.837 ± 0.021*
%CHO	44.6 ± 5.1	44.1 ± 7.3	55.3 ± 6.0	46.7 ± 6.9*
%FAT	55.4 ± 5.1	55.9 ± 7.3	44.7 ± 6.0	53.3 ± 6.9*
g CHO/min	0.70 ± 0.12	0.69 ± 0.14	1.08 ± 0.20	0.97 ± 0.22*
g FAT/min	0.33 ± 0.02	0.33 ± 0.03	0.33 ± 0.03	0.40 ± 0.03*
kcal/min	5.74 ± 0.42	5.71 ± 0.38	7.10 ± 0.58	7.39 ± 0.67

Different from follicular *p<0.025 (α=0.05), **p<0.01

Plasma glucose concentrations

There were no significant differences in glucose concentrations (Table 7) between the males and females, although glucose concentrations tended to be higher in the luteal and follicular females (4.39 and 4.32 mM, respectively) compared to the males (3.94 mM) at the low intensity (p=0.075 and p=0.043 > 0.017 (α=0.05), respectively). Glucose concentrations were slightly but significantly lower

(~0.13 mM) in the luteal phase during rest, low intensity, and high intensity workloads (Table 8).

Table 7. Glucose concentrations during the two-stage subthreshold trial gender comparison. Expressed as mean ± standard error.

	Males	Follicular	Luteal
Rest	4.19 ± 0.20	4.23 ± 0.09	4.42 ± 0.10
Low	3.94 ± 0.33	4.32 ± 0.15	4.39 ± 0.15
High	4.49 ± 0.21	4.49 ± 0.12	4.50 ± 0.16

Different from males *p<0.017 (α=0.05), **p<0.01

Table 8. Glucose concentrations (mM) during the two-stage subthreshold trial menstrual phase comparison. Expressed as mean ± standard error.

	Follicular	Luteal
Rest	4.28 ± 0.08	4.42 ± 0.10*
Low	4.23 ± 0.17	4.39 ± 0.15**
High	4.38 ± 0.16	4.50 ± 0.16*

Different from follicular *p<0.033 (α=0.05), **p<0.01

Plasma glucose kinetics

Only three of the five luteal trials used for all other gender comparisons were available for comparison of plasma glucose kinetics because of implausible results from gas chromatography-mass spectrometry analysis. Since there was a significant difference in body composition (i.e., body fat percentage), the glucose rates of appearance (Ra) and disappearance (Rd) were expressed and compared as umol/kg/min and umol/kg lean body mass/min. Glucose concentrations were stable at rest in all subjects, therefore glucose rates of appearance and disappearance (i.e., glucose turnover) into and out of the circulation were equal (i.e., Ra=Rd at rest). During rest and the low intensity workload, there were no differences in the

glucose rates of appearance or disappearance between the males and females regardless of the units used to express the kinetics (Tables 9-12).

At the high intensity workload, Ra (umol/kg/min) tended to be lower (24.0 vs. 30.0) in the follicular females ($p=0.026 > 0.017$ ($\alpha=0.05$) (Table 9)) versus the males. However, when expressed as Ra (umol/kg lean body mass/min) follicular females were similar to males (28.8 vs. 32.9, $p=0.16$) (Table 10). Rd (umol/kg/min) also tended to be lower (25.4 vs. 29.8) in the follicular females ($p=0.056 > 0.017$ ($\alpha=0.05$)) (Table 11), but when Rd was expressed as umol/kg lean body mass/min follicular females and males were similar (30.4 vs. 32.7 (Table 12)). At the high intensity, regardless of the units used to express glucose turnover, the luteal phase females had significantly lower rates of glucose turnover compared to the males (Tables 9-12).

Table 9. Glucose rates of appearance (Ra) into the circulation (umol/kg/min) during the two-stage subthreshold trial gender comparison. n=5 males, n=5 follicular females, n=3 luteal females. Expressed as mean ± standard error.

	Males	Follicular	Luteal
Rest	12.1 ± 0.2	12.9 ± 0.6	12.5 ± 0.2
Low	19.3 ± 1.9	17.1 ± 1.7	16.6 ± 1.3
High	30.0 ± 3.7	24.0 ± 2.7	20.0 ± 3.2**

Different from males *p<0.017 (α=0.05), **p<0.01

Table 10. Glucose rates of appearance into the circulation (umol/kg lean body mass/min) during the two-stage subthreshold trial gender comparison. n=5 males, n=5 follicular females, n=3 luteal females. Expressed as mean ± standard error.

	Males	Follicular	Luteal
Rest	13.4 ± 0.4	15.4 ± 0.5	14.7 ± 0.2
Low	21.1 ± 1.9	20.5 ± 2.0	19.5 ± 1.5
High	32.9 ± 3.9	28.8 ± 3.4	23.5 ± 3.9*

Different from males *p<0.017 (α=0.05), **p<0.01

Table 11. **Glucose rates of disappearance out of the circulation (umol/kg/min) during the two-stage subthreshold trial gender comparison. n=5 males, n=5 follicular females, n=3 luteal females. Expressed as mean ± standard error.**

	Males	Follicular	Luteal
Rest	12.1 ± 0.2	12.9 ± 0.6	12.5 ± 0.2
Low	17.9 ± 1.7	16.3 ± 1.4	15.8 ± 1.2
High	29.8 ± 3.3	25.4 ± 2.6	19.4 ± 6.5**

Different from males *p<0.017 (α=0.05), **p<0.01

Table 12. **Glucose rates of disappearance (umol/kg lean body mass/min) during the two-stage subthreshold trial gender comparison. n=5 males, n=5 follicular females, n=3 luteal females. Expressed as mean ± standard error.**

	Males	Follicular	Luteal
Rest	13.4 ± 0.4	15.4 ± 0.5	14.7 ± 0.2
Low	19.6 ± 1.7	19.5 ± 1.7	18.6 ± 1.6
High	32.7 ± 3.5	30.4 ± 3.1	22.7 ± 4.2**

Different from males *p<0.017 (α=0.05), **p<0.01

For the glucose kinetics menstrual phase comparison, data from only 4 of the 5 females were available due to implausible results obtained from gas chromatography mass spectrometry analysis. During rest and the low workload, there were no differences in glucose kinetics between the luteal and follicular phases. At the high workload there were no differences in Ra (umol/kg/min) between follicular and luteal phases (Figure 2). However, rates of glucose disappearance and clearance (Rc= Rd/[Glucose]) were significantly lower during the high workload of the luteal phase (Figures 3 and 4).

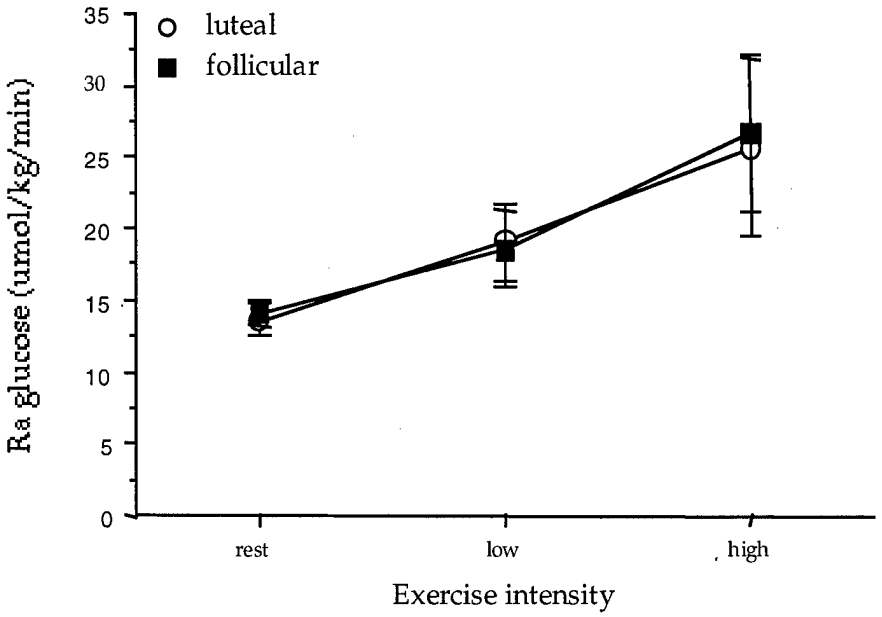

Figure 2. **Glucose rates of appearance (Ra) into the circulation during the two-stage subthreshold trial menstrual phase comparison. Expressed as mean ± standard error. *p<0.033**

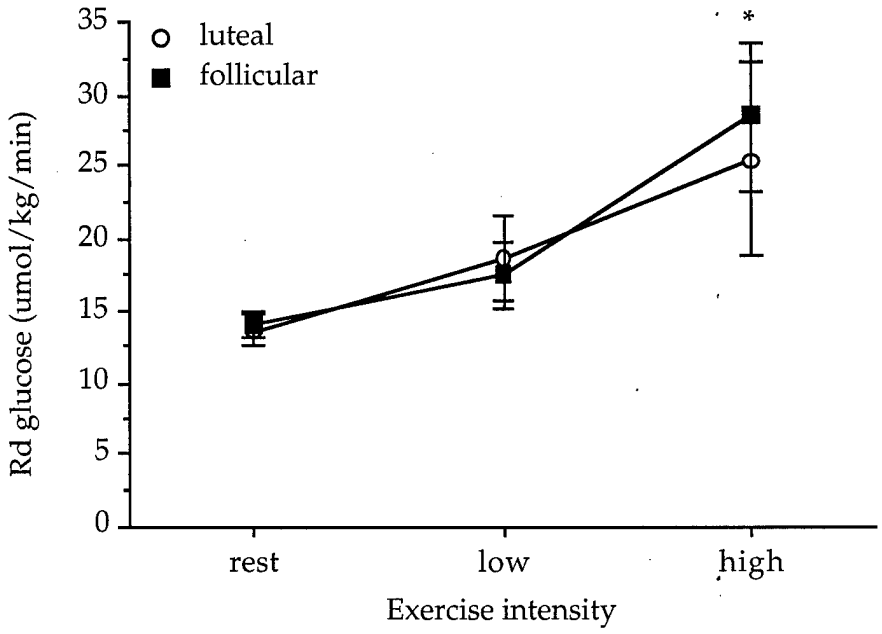

Figure 3. **Glucose rates of disappearance (Rd) out of the circulation during the two-stage subthreshold trial menstrual phase comparison. Expressed as mean ± standard error. *p<0.033**

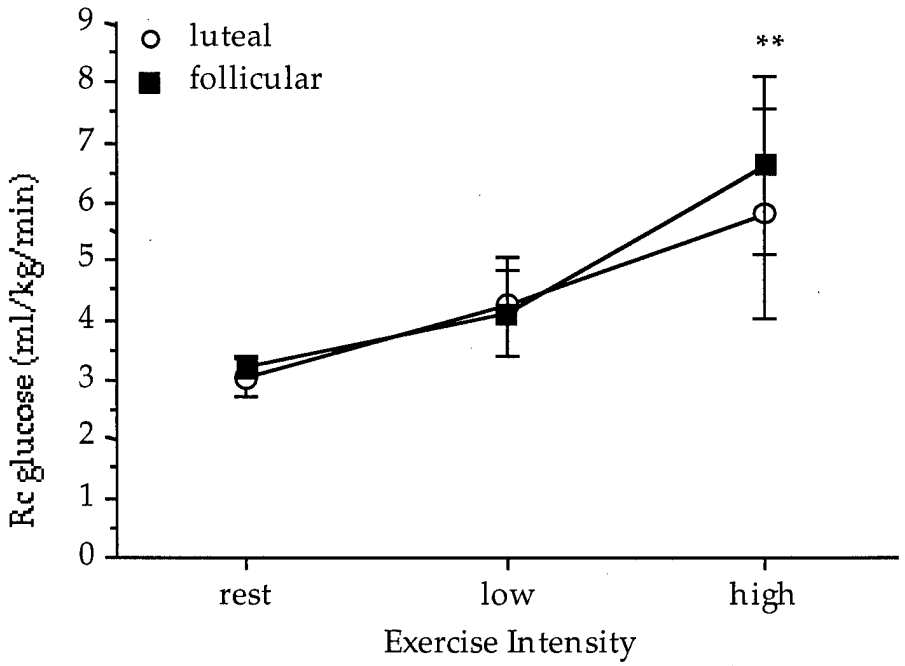

Figure 4. Glucose clearance rate (Rc) during the two-stage subthreshold trial menstrual phase comparison. Expressed as mean ± standard error. **p<0.01

Plasma lactate concentrations

There were no differences in plasma lactate concentrations between males and either the follicular or luteal phase females during rest and the low intensity (Table 13). However, at the high workload, males had significantly higher plasma lactate concentrations than both phases of the females.

Table 13. Plasma lactate concentrations (mM) during the two-stage subthreshold trial gender comparison. Expressed as mean ± standard error.

	Males	Follicular	Luteal
Rest	1.52 ± 0.14	1.51 ± 0.19	1.22 ± 0.10
Low	2.04 ± 0.24	2.26 ± 0.12	1.68 ± 0.14
High	4.32 ± 0.67	3.24 ± 0.34*	2.48 ± 0.41*

Different from males *p<0.017 (α=0.05), **p<0.01

There were no differences in plasma lactate accumulation during rest and the low intensity between the follicular and luteal phases (Table 14). At the high workload, there was a greater plasma lactate concentration during the follicular phase ($p<0.017$).

Table 14. Plasma lactate concentrations (mM) during the two-stage subthreshold trial menstrual phase comparison. Expressed as mean ± standard error.

	Follicular	Luteal
Rest	1.49 ± 0.20	1.22 ± 0.10
Low	2.01 ± 0.15	1.68 ± 0.14
High	3.08 ± 0.39	2.48 ± 0.41*

Different from males *p<0.017 (α=0.05), **p<0.01

Insulin

The luteal females had significantly higher insulin concentrations than males during rest, low and high workloads (Table 15). The follicular females had significantly higher insulin concentrations during rest and at the high workloads, while insulin tended to be higher during the low workload (3.88 vs 4.72 mIU/ml, $p=0.023>0.017$ ($\alpha=0.05$)).

There were no significant differences in plasma insulin concentrations between follicular and luteal phases (Table 15), although insulin tended to be lower at the high intensity during the luteal phase (4.11 vs 4.95 mIU/ml, $p=0.089>0.033$ ($\alpha=0.05$)).

Table 15. Insulin concentrations (mIU/ml) during the two-stage subthreshold trial gender comparison. Expressed as mean ± standard error.

	Males	Follicular	Luteal
Rest	4.19± 0.42	5.45 ± 0.61	5.17 ± 0.33
Low	3.88 ± 0.51	4.72 ± 0.32	4.72 ± 0.16
High	3.49 ± 0.39	4.95 ± 0.36	4.11 ± 0.52

Different from males *p<0.017 (α=0.05), **p<0.01

Table 16. Insulin concentrations (mIU/ml) during the two-stage subthreshold trial menstrual phase comparison. Expressed as mean ± standard error.

	Follicular	Luteal
Rest	5.22 ± 0.63	5.07 ± 0.16
Low	4.47 ± 0.37	4.72 ± 0.16
High	4.73 ± 0.53	4.11 ± 0.52

Estimation of plasma glucose, muscle glycogen, and fat to total substrate oxidation

While the follicular phase females tended to contribute more of their blood glucose to total carbohydrate oxidation (Figure 5) there were no significant between males and follicular females at either intensity, nor between males and luteal phase females (Table 17).

Table 17. Percentage of blood glucose contribution to total carbohydrate oxidation. Expressed as mean ± standard error.

	Males	Follicular	Luteal
Low	22.3 ± 2.8	29.2 ± 4.8	24.4 ± 3.5
High	21.5 ± 2.5	26.4 ± 2.8	21.6 ± 4.2

Different from males *p<0.017 (α=0.05), **p<0.01

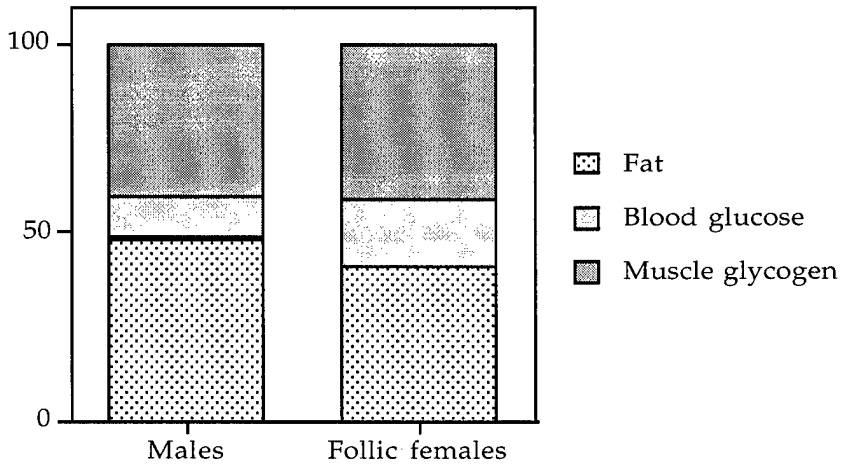

Figure 5. Relative contributions of fat, blood glucose, and muscle glycogen to total substrate oxidation during the high workload gender phase comparison. Blood glucose contribution to total energy expenditure tends to be greater in follic females ($p<0.01$).

Plasma glucose contributed 37% of the total carbohydrate oxidized during the luteal phase and 33% during the follicular phase at both intensities (Figure 6). Muscle glycogen oxidation contributed conversely 63% and 67% to total carbohydrate oxidation, respectively. There was no significant difference between phases in the percent contributions. When expressed as the percent contribution of plasma glucose to total kilocalories expended, plasma glucose during the follicular phase contributed slightly more to energy expenditure than during the luteal phase (16.4% vs 14.2%, $p=0.032>0.025$ ($\alpha=0.05$)). There were no significant differences in muscle glycogen oxidation between follicular and luteal phases (2.66 vs 2.54 kcal/min, at the high workload, respectively, $p=0.38$), nor was there a difference at the low workload (1.67 vs 1.75 kcal/min, $p=0.55$).

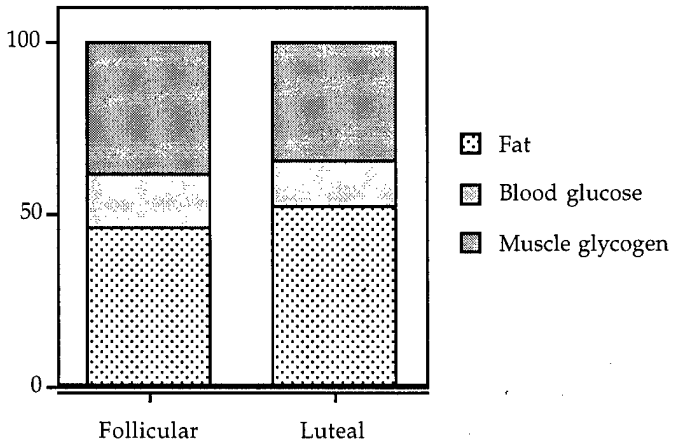

Figure 6. Relative contributions of fat, blood glucose, and muscle glycogen to total substrate oxidation during the high workload menstrual phase comparison. Blood glucose utilization is higher in follicular phase ($p<0.023$).

The purpose of this study was to compare carbohydrate and fat metabolism in males and females during exercise below their respective lactate thresholds. The subpurpose of this study was to determine if females metabolize carbohydrate and fat similarly during the follicular and luteal phases of the menstrual cycle. The subjects were studied while working at exercise intensities (~70%LT and ~90%LT) below the lactate threshold for two purposes: 1) to insure that the respiratory exchange ratio (e.g., VCO2) represented tissue CO2 production and was not influenced by acid buffering by bicarbonate pools, and 2) to maintain the potential effects of reproductive hormones on the glucoregulation capacity of pancreatic hormones. At higher intensities (e.g., above LT), the pancreatic hormones apparently play little role in controlling rates of hepatic glucose production and the

factors that control glucose production at high intensities remain unidentified (Coggan et al., 1997).

Substrate oxidation in males and females

The results of this study suggest that conclusions about gender differences in substrate oxidation depend upon the exercise intensity and the menstrual phase during which the comparison females are tested. The decision to compare males and females with respect to their lactate threshold instead of the more traditional approach of selecting exercise intensities with respect to VO_{2max} was based on the observation that the lactate threshold is a better measure or predictor of substrate oxidation. Subjects with the same VO_{2max} and different thresholds can have vastly different rates of carbohydrate and fat oxidation during moderate intensity exercise (Coggan et al, 1992). Kanaley et al (1995) has demonstrated that moderately trained runners and marathon runners with significantly different VO_{2max} values of 52 and 65 ml/kg/min have similar respiratory exchange ratios (RER) at intensities just above and below their respective lactate threshold. Similarly, Pereira & Freedson (1997) have demonstrated that trained runners and moderately trained runners (69 and 58 ml/kg/min, respectively) have similar RER values at ~88% of the VO_2 at the lactate threshold. Due to the apparent association of the lactate threshold with substrate oxidation (i.e., RER), this study was designed to have males and females exercise at intensities relative to their respective lactate thresholds.

The males and follicular females of this study oxidized similar proportions of fat and carbohydrate at both intensities examined in this study. These results are in contrast to the findings of Froberg & Pedersen (1984), Jansson (1986), Tarnopolsky et

al. (1990), and Tarnopolsky et al. (1995), but are in accord with the results of Costill et al. (1979), Wallace et al, and Mendenhall et al (1995). The studies that were in conflict with the results of this study did not control for the lactate threshold and the explanation for the increased fat oxidation in the female subjects may have been due to greater metabolic fitness (i.e., higher lactate threshold).

The discrepancy between these results and Tarnopolsky et al (1990) who controlled for diet may have been due to the effect of the macrocomposition of the pre exercise trial diets. Aside from the 10 hour fast before all trials, the diet of the subjects was not controlled. The males tended to consume more kilocalories (45 vs 29 kcal per kg,$p=0.06$). However, the percentage of calories derived from carbohydrates was significantly greater in the females (73 vs 54%, $p<0.01$) and consequently there were no differences in carbohydrate intake (5.8 vs 6.2 g/kg, females vs males $p>0.6$) between males and females. Interestingly, Tarnopolsky et al. (1995) demonstrated that follicular females who increased their carbohydrate intake from ~55% to ~75% of total kilocalories ingested had no significant effects on muscle glycogen concentration or utilization during exercise. Lambert et al (1994) demonstrated that a high fat diet (70% fat, 7% carbohydrate) compared to a low fat diet (74% carbohydrates, 12% fat) significantly reduces RER values during moderate intensity exercise. These are extreme diets and whether the differing diets between the males and females in the present investigation affected RER values remains unknown. If the composition of the diet did indeed favor an increased carbohydrate oxidation in the females, this would explain the disparity between the results of Tarnopolsky et al (1990 and 1995) and the present male vs follicular

comparison. However, the differences in diets between the males and the luteal females improves the likelihood that luteal phase females oxidize proportionally less carbohydrate than males, since there is decreased carbohydrate oxidation ($p=0.025 > 0.017$ ($\alpha=0.05$)) in the luteal females even though they consumed a greater proportion of their kilocalories as carbohydrates. Future gender comparison studies should control for the macrocomposition of the diet to eliminate this speculation.

Gender comparison of blood glucose production (Ra)and utilization (Rd)

During rest and the low workload there were no differences between males and the females in glucose rates of appearance (Ra) or rates of disappearance (Rd). At the high workload the conclusion about gender differences in glucose Ra and Rd depends upon the units used to express these parameters and the menstrual phase of the females. While Ra and Rd (umol/kg/min) appeared to be lower in the follicular females, when expressed relative to lean body mass (i.e., umol/kg LBM/min) the males and follicular females were similar. Since glucose uptake by the adipose tissue during exercise is most likely negligible compared to the glucose Rd of active skeletal musculature, factoring out adipose tissue probably gives a better representation of the glucose kinetics in two groups who are significantly different in body composition. Since the follicular females have similar rates of glucose utilization (Rd) to the men and their absolute workload (e.g., total kilocalories expended was lower) , they relied significantly more on blood glucose for their total energy expenditure at both the low and the high workloads (11.5% vs 8.8% and 14.2% vs 11.0%, respectively, $p<0.001$). The follicular females also tended to have greater relative contributions of blood glucose to total carbohydrate

oxidation at the low and high workloads (29.2% vs 22.3%, p=0.07 and 26.4% vs 21.5%, p=0.17, respectively). This finding is in agreement with the only other study that has compared males and follicular females with glucose stable isotope tracers (Mendenhall et al., 1995). They determined that blood glucose contributed a higher percentage to total carbohydrate oxidation than in the males (28 vs 20%, p<0.001). And conversely, muscle glycogen contribution to total carbohydrate oxidation was less in the females (72 vs 80%, p<0.001). They speculated that the two fold greater epinephrine levels observed in their male subjects were responsible for the greater contribution of muscle glycogen to total carbohydrate oxidation. Since epinephrine concentrations were not measured in the present investigation, conclusions about this mechanism cannot be made.

Regardless of the expression of glucose kinetics, luteal females had decreased glucose Ra and Rd compared to the males. Males and luteal females contribute similar proportions of their muscle glycogen and blood glucose to total carbohydrate oxidation. However, as there were only 3 luteal females available for these comparisons, conclusions about males and luteal females are surely tenuous at this point.

Gender comparison of insulin, glucose, and lactate

In agreement with Tarnopolsky et al (1990), males had lower insulin concentrations during rest, low, and high workloads regardless of menstrual phase. It may be possible that this decreased insulin concentration was due to greater male epinephrine levels as determined by Tarnopolsky et al, Mendenhall et al (1995), Graham et al (1986), and Nygaard (1981) and suggested by significantly greater lactate

concentration (4.3mM males vs 3.2 mM follicular, vs 2.5 mM luteal, $p<0.01$) in the males at the high workload. Despite the differences in insulin concentrations, there were no differences between males and females in glucose concentrations which is in agreement with Jansson (1986) who exercised males and females for 25 minutes at 65%VO2max. While Tarnopolsky et al (1990) observed lower blood glucose concentrations in males, it is possible that the males of that study had lower lactate thresholds and an associated greater glucose Rd (Coggan et al., 1992).

Substrate oxidation across the menstrual cycle

The primary finding from this component of the study is that carbohydrate oxidation is decreased and fat oxidation is increased during the luteal phase of the menstrual cycle which is in agreement with Hackney et al. (1994) and Dombovy et al. (1987), and suggested by others data (Hirata et al., 1986). Hackney et al. (1994) used respiratory exchange ratio (RER) and VO_2 to measure carbohydrate and fat oxidation during the mid follicular and mid luteal phases in 9 women with a mean VO_{2max} of 46.0 ml/kg/min during treadmill running. At 35% and 60%VO2max, the mean RER was significantly lower during the luteal phase and thus carbohydrate oxidation was reduced and fat oxidation increased during the luteal phase. While there was no statistical difference in carbohydrate or fat oxidation, RER was still slightly higher during follicular phase at 75% VO_{2max}. The authors concluded that exercise at 75% VO_{2max} requires predominantly carbohydrate regardless of phase. Dombovy et al., also observed decreases in RER during an incremental VO_{2max} test in the luteal phase. However, in accord with the results of the current study, there

were no differences in RER at the lower workloads (40-50% VO2peak) but differences in RER at intensities above 67% VO_{2max}.

In agreement with our results and Dombovy et al., Hirata et al. (1986) also determined that in females with similar VO_{2max} values to our subjects, exercise at 40% VO_{2max} (i.e., also the low workload of the present investigation) elicited similar mean RER values during follicular and luteal phases (0.788 vs 0.783, respectively) while at 70%VO2max their mean RER was lower during the luteal phase (0.849 to 0.827, respectively, $p>0.05$) although not statistically lower. This discrepancy in statistical results between their study and the present investigation may be explained by the statistics used to compare the phase responses. Hirata et al. (1986) used the statistical paired t-test to detect differences while in this study the more powerful *a priori* planned comparisons were used to detect differences. Kanaley et al. (1992) and Nicklas et al. also concluded that there were no differences in substrate oxidation between follicular and luteal phases. The subjects of Kanaley et al. (1992) were of greater aerobic fitness than the subjects of the current investigation (48 vs 57 ml/kg/min VO2max), but still had consistently (not statistically) lower RER values for 90 minutes of exercise in the luteal phase. The subjects of Nicklas et al. had significantly lower RER values during the luteal phase at rest (0.86 to 0.71, $p<0.05$) but during 90 minutes of exercise at the relatively high intensity of 70% VO_{2max} there were no apparent differences.

Menstrual phase and blood glucose utilization

While previous menstrual phase studies have relied exclusively on RER to determine substrate oxidation, this is the first study to have studied blood glucose

uptake during exercise across the menstrual cycle. In accord with the 10% decrease in carbohydrate oxidation (RER and VO_2) during the luteal phase, there was a concomitant 11% decrease in glucose uptake from the circulation. Assuming that 100% of the glucose Rd is oxidized at 50% VO_{2max} (Jeukendrup et al., 1997) and menstrual phase does not affect the proportion of Rd that is oxidized, muscle glycogen utilization must have also been decreased by a similar percentage during the luteal phase.

Ruby et al. (1997) also observed a decrease in glucose Rd in amenorrheics treated with estradiol. In contrast to this study, there were no significant differences in total carbohydrate or fat oxidation as determined by RER. They also determined a lower glucose Ra which they attributed to the suppressive effect of estradiol on gluconeogenesis previously demonstrated in rats (Ahmed & Bailey, 1981; Sladek et al., 1974). The differences in the Ra and RER responses between Ruby et al. (1997) and the current investigation are most likely due to the low levels of circulating progesterone in amenorrheics. Indeed, progesterone is known to antagonize estradiol suppression of gluconeogenesis (Ahmed & Bailey, 1974) and estradiol promoted fat oxidation in rats (Hatta et al, 1990). As suggested by Reinke et al. (1972) and Bisdee et al. (1988), the ratio of estradiol to progesterone may be the most important variable for dictating the metabolic response. Since this study did not investigate the level of circulating progesterone, no conclusions about this ratio can be made.

The decrease in the glucose uptake (Rd) during the luteal phase at the higher workload may have been due to the associated decrease in insulin concentration

(p=0.09) and or decreased insulin sensitivity during the luteal phase (Diamond et al., 1989; Elkind-Hirsch et al., 1993). As insulin facilitates Rd during exercise (Gao et al., 1994) decreased insulin concentration and or action could explain the decreased glucose Rd at the higher workload during the luteal phase.

The greater lactate concentration in the follicular phase is also in accord with the greater carbohydrate oxidation and glucose Rd observed during the follicular phase. McCracken et al. (1994) also reported that the lactate during recovery from ~30 minutes of exhaustive exercise was higher (8.7 vs 5.4 mM, $p<0.05$) during the follicular phase. The increased lactate concentration at the high workload during the follicular phase was most likely due to a greater glycolytic flux and not due to reduced lactate removal as Jurkowski et al. (1981) determined that there were no differences in the clearance of lactate during exercise.

It is possible that the shift from carbohydrate oxidation to fat oxidation during the luteal phase was due to an increase in free fatty acid (FFA) availability. Reinke et al. (1972) observed that resting FFA levels were significantly higher during the mid luteal phase compared to the follicular and ovulation phases. Elevated estradiol (178 vs 71 nM, p=0.09) and or increased growth hormone concentrations (Nicklas et al., 1989) in the luteal phase may be mediating increased lipolytic action. This proposed mechanism for decreasing carbohydrate oxidation would be in agreement with Hargreaves et al. (1991) who found a decrease in glucose uptake by leg muscles when FFA levels were increased from 0.6 mM to greater than 1.0 mM with the infusion of a triglyceride emulsion (Intralipid) and heparin. Bracy et al. (1995) also demonstrated a decreased blood glucose uptake by increasing FFA levels from 0.3 to

1.0 mM in dogs. During the lower workload of this present study, FFA availability may not have been as limiting because the demand for substrate was not as great. Bonen et al. (1983) and Bonen et al. (1991) reported no menstrual phase differences in FFA levels during light (35-40%VO2max) and heavy exercise (83-85%VO2max) which were intensities below and above the intensity (~53%VO2max) where we have observed differences in fat and carbohydrate oxidation. Without FFA concentrations and kinetics, the proposed mechanism for decreased blood glucose utilization and carbohydrate oxidation is speculative.

Another explanation for the decreased carbohydrate oxidation would be decreased muscle glycogen stores. Rates of muscle glycogenolysis and carbohydrate oxidation are directly related to muscle glycogen concentration (Hargreaves, 1997). Therefore, if pre-exercise muscle glycogen was greater during the follicular phase this could explain the increased carbohydrate oxidation and lactate concentration. However, to avoid differences in pre-exercise muscle glycogen, subjects were asked to replicate their diet two days preceding each trial and to refrain from exercise 36 hours before each trial. Post-hoc comparison of diet records between follicular and luteal phases indicate that there were no phase differences in energy intake (30.7 vs 29.3 kcal/kg, respectively, $p=0.7$) or carbohydrate intake (5.9 vs 5.2 g/kg CHO, respectively, $p=0.31$).

While energy balance (i.e., dietary intake minus energy expenditure) was assumed to be similar between phases due to similarities in dietary intake and physical activity this does not preclude differences in resting energy expenditure. Bisdee et al. (1989) observed a significantly greater resting energy expenditure of 130

kcal/day during the luteal phase, while Eck et al. (1997) and Piers et al. (1995) have reported that resting energy expenditure is similar during the luteal and follicular phases. The gross marker of energy balance, body weight, was significantly higher (60.5 to 60.0 kg, $p<0.05$) during the luteal phase suggesting maintenance of positive energy balance. However, water retention may have been responsible for the increased body weight. If so, then it may be possible that during the luteal phase the females were actually in negative energy balance (provided they retained one kilogram or more of water) and the decreased carbohydrate oxidation could have been due to a relative depletion of muscle glycogen. If reduced energy intake of carbohydrates was responsible for the decline in carbohydrate oxidation we would expect to see a positive relationship between carbohydrate intake and oxidation between our subjects. In contrast, there is a strong negative relationship ($r= -0.96$, $p<0.01$) between carbohydrate intake and oxidation during the luteal phase. That is, during the luteal phase the females who consumed less carbohydrates per kilogram oxidized proportionally more carbohydrates during the high workload. Furthermore, in both of the studies that have compared resting muscle glycogen, muscle glycogen was found to be higher during the luteal phase (Hackney, 1990; Nicklas et al., 1989). Nicklas et al. determined that repletion was greater after depleting exercise during the luteal phase even though dietary intake (as in this study) was slightly lower during the luteal phase (1691 vs 1813 kcal/day $p>0.05$). Nevertheless, future studies in this area should measure resting energy expenditure during the menstrual phase of interest and adjust dietary intake accordingly.

There are apparently no studies that have found that carbohydrate oxidation is higher during the luteal phase. While this study does not prove the converse, from a meta analysis point of view, if there were indeed no differences between phases and bias did not influence experimental designs and results, there should be a similar number of studies that find decreased carbohydrate oxidation and the converse, increased carbohydrate oxidation during the luteal phase.

Conclusions

In conclusion, these results suggest that there are menstrual phase differences in substrate oxidation patterns in recreationally active females. Conclusions about gender differences in substrate oxidation depend upon the exercise intensity and menstruation status of the female. With the present data, it cannot be determined whether the differences in substrate oxidation are due to increased lipid availability during the luteal phase or a decreased glycolytic flux due to decreased muscle glycogen concentrations. Future research in this area should employ stable isotope FFA tracers to determine the source of the increased fat oxidation (i.e., plasma FFA and/or intramuscular triglycerides) witnessed during the luteal phase. The muscle biopsy technique should also be utilized to determine pre-exercise glycogen concentrations. Studies that examine the effects of diet, training, and exogenous substances on substrate utilization should recognize and take into account the menstrual status of their female subjects.

Problems with data collection

There were no problems associated with the collection of data for this component of the study.

II - Effects of menstrual function, gender, total energy expenditure (TEE), and the maintenance of energy balance on measures of bone health in males and females.

Purpose:

The purpose of this component of the project has been to determine the relationship between menstrual function, gender and total energy expenditure (TEE) on measures of bone health in males and females exposed to seasonal prolonged arduous work (extended operations). The proposed directional hypotheses for this component are listed below.

Objectives and Assumptions

Because the increased recruitment and possible interests of females in occupational settings that involve frequent exhausting physical exertion, the energy requirement for this population warrants additional investigation. The relationship between energy expenditure (training mileage) has been loosely related to menstrual dysfunction in active or endurance trained females (Boyden et. al., 1983; Bullen et. al., 1985; Shangold et. al., 1979). At best, menstrual function in response to a physically demanding occupational setting has been described as *similar* (Jeyaseelan & Rao, 1995).

The shortcomings of a *simulated* testing scenario may decrease the expected physical and mental stress associated with the actual occupational setting. Therefore, in the proposed model, wildland firefighters assigned to "active duty" served as subjects. This subject population was subjected to "real", un-simulated occupational demands and hazards, similar to the physical demands of the soldier in rigorous combat situations. It is the purpose of this investigation to evaluate the

energy expenditure and energy intake of male and female wildland firefighters so as to provide them with the necessary medical care and protection against any health concerns associated with menstrual dysfunction and risk for bone related injuries. If a decrement in energy intake exists, the ramifications on overall health and ultimately safety may be extreme. Females subject to arduous physical environments may have truly unique nutritional concerns compared to their male co-workers. Based on a conservative estimate of energy expenditure for typical wildland firefighting tasks (7.5 kcals/min), during a difficult 12-14 hour work day, total energy expenditure may exceed 4050 to 4725 kcals/12 and 14 hours, respectively (assuming 45 minutes per hour of work). Not only might the nutritional demand exceed the dietary intake, but the effects on menstrual function and seasonal bone related injuries may be exponential.

Hypotheses and Expected Results

1. It is expected that the energy expenditure due to occupational demands will be in excess to the dietary energy intake, therefore resulting in a state of negative energy balance.

2. If negative energy balance results due to occupational demands, this will be associated with an increase in reported menstrual cycle irregularity and increases in bone resorption.

3. It is expected that negative energy balance and increased bone resorption will be more prominent in the female subjects.

Procedures

Subject Recruitment

Subjects included male and female wildland firefighters recruited from two Interagency Hot Shot Crews (Lolo and Bitterroot crews) from the Missoula area. Subjects were recruited through an informative meeting arranged between the Principal Investigator and all Interagency Hot Shot Crew Supervisors in mid May, 1997. After this meeting, crew Supervisors solicited crew members for potential subjects. An informational meeting was then arranged between the Principal Investigator and the entire crew. At this time, the objectives of the study and the outline of data collection was discussed. Upon selecting eight volunteers from each crew (n=4 males, n=4 females from each crew) pre-season testing was scheduled.

Preliminary Screening and Pre-season Testing:

Prior to data collection, all subjects read and signed an Internal Review Board (IRB) approved human subject's consent form as identified in Appendix VI of the Broad Agency Announcement. Subjects completed a detailed health history to determine prior exercise and training habits and menstrual regularity. At the initial pre-season testing appointment, subjects produced a "clean catch" urine sample for the determination of pre-season bone resorption characteristics using a cross-linked N-telopeptides (NTx) assay.

Body composition

Body fat and lean body mass was assessed by hydrostatic weighing at estimated residual lung volume. Residual lung volume was estimated using the prediction equations established by Boren et al., (1966) and Black et al., (1974). Percent body fat

was calculated from body density using a Lohman (1992) age/gender specific equations.

Exercise testing

Each subject also completed a multi-stage maximal exercise test using a motorized treadmill (Quinton Q65, Seattle, WA). All metabolic testing information was collected using a TEEM 100 (Aerosport Inc., Ann Arbor, MI) metabolic system equipped with a medium-flow (10-120 l/min) pneumotach. Prior to each treadmill test, the metabolic system was gas calibrated with known concentrations of O_2 and CO_2, and flow rate was calibrated with a 3 l syringe according to the manufacturer (Aerosport Operators Manual, 1993). Heart rate was continuously monitored using a telemetry chest strap heart rate monitor for all testing (Polar, Port Washington, NY).

Pre-season data was also collected outside the Human Performance Laboratory at the Western Montana Clinic. Subjects were transported to the clinic for measures of site-specific bone mineral content using dual energy X-ray absorbtiometry (DEXA) unit. Bone densitometry was performed using the Lunar DPX-IQ scanner at the femoral neck and lumbar site locations.

Seasonal Data Collection:

During the 1997 fire season, periodic urine samples were obtained for subsequent determination bone resorption using the NTx assay as mentioned above (see Figure 7) . Female subjects were also asked to document menstrual cycle activity throughout the duration of the fire season, indicating specific days of menstruation and any sexual activity.

Seasonal Collections				
Time Points	Pre-season	Early-season	Mid-season	Late-season
n=6 females	X	X	X	X
n=4 males	X	X	X	X

Acute Collections During TEE Measurement Period					
Time Points	Pre-season	Day #0	Day #2	Day #3	Day #5
n=3 females	X	X	X	X	X
n=2 males	X	X	X	X	X

Figure 7. Sample collection time points for the measure of bone resorption (nM BCE/mM creatinine). Samples were collected to evaluate seasonal and acute changes in bone resorption.

Energy Expenditure Measurements:

Energy expenditure during simulated, job specific laboratory testing was performed using indirect calorimetry and the above mentioned metabolic system. Prior to the maximal treadmill testing, eight subjects completed a multi-stage, submaximal test using a treadmill apparatus that simulates fire line building. In combination with the data collected during the initial three workloads of the treadmill test, two linear regression lines were developed for each subject that completed both the treadmill and line building protocols. These equations were then used in conjunction with field measures of heart rate and activity inventories to estimate daily energy expenditure during work operations.

During the 1997 fire season, five subjects were selected for measures of total energy expenditure (TEE) during extended field operations. These subjects were provided a dose of doubly labeled water (0.39g/kg BW $H_2{}^{18}O$, 0.23g/kg BW ^2H_2O - Cambridge Isotope Laboratories, Andover, MA). Subjects were given the initial dose of doubly labeled water upon arrival to the incident in the evening hours prior to

initiating fire suppression activity. The protocol for the dosing and the daily collection schedule is detailed below in Figure 8. Although the initial dose is somewhat higher (for $H_2{}^{18}O$) compared to estimates suggested by Wolfe, 1992 (0.25g/kg BW $H_2{}^{18}O$, 0.3g/kg BW ^2H_2O), a larger dose is warranted to compensate for the higher rates of water turnover (expected in these subjects). In addition to the dose of doubly labeled water, a second dose of ^2H_2O (approximately 2.0g) was administered on the eve of day 5 after the collection of a second background measure. The following morning, a final urine sample was collected to adjust changes in total body water (observed during the TEE measurement period) for variations in total body water.

Day	-1	0	1	2	3	4	5	6
Collection time	2100	0430	0430	0430	0430	0430	0430	0430
Dose Information								
0.39g $H_2{}^{18}O$, 0.23g ^2H_2O/kg BW	*							
2.0 g ^2H_2O							*	
Sampling information								
($H_2{}^{18}O$ - †, ^2H_2O - ¥)	(†, ¥)	(†, ¥)	(†, ¥)	(†, ¥)	(†, ¥)	(†, ¥)	(†, ¥)	(†, ¥)
urine - *	*	*	*	*	*	*	*	*
nude BW - Δ		Δ			Δ			Δ
NTx samples - ß	ß	ß	ß	ß	ß	ß	ß	ß
Calculations								
Total body water (TBW)	TBW							TBW
Total energy expenditure (TEE)		< TEE collection/measurement period >						

Figure 8. Dose and daily collection schedule for measures of TEE using the doubly labeled water technique.

Dietary Intake/Food Records:

Dietary intake during the seasonal measures was completed using a detailed food record/ inventory of all ingested materials. When subjects were accessible in camp, tray surveys and measures of pre and post food weights were used. However, this was only possible for some dinner and breakfast times.

Results and Discussion

Subject characteristics

Descriptive data for all subjects are presented in Table 18. It is difficult to comment fully on differences noticed during the season due to a limited sample size (not all subjects have completed post testing at this time). However, males showed a significant decrease in percent body fat whereas seasonal activity did not alter the females percent body fat. Although there was no apparent change in BMD (L1-L4), females (those who have been post tested, n=4) thus far show a statistically significant decrease in BMD (femoral neck) as a result of seasonal operations. However, samples for bone resorption (NTx) have not been assayed at this. Therefore it is difficult to speculate why there may have been site specific bone mineral loss.

Table 18. Descriptive data for the sample of wildland firefighters studied during the 1997 fire season.

Variable	MALES		FEMALES	
	Pre-Season	Post-Season	Pre Season	Post Season
Body Weight (kg) (n=7 M, 5 F)	71.6±1.8	70.3±1.5	63.6±2.9	63.3±4.9
Percent Body Fat (n= 7 M, 5 F)	12.5±1.8	9.5±1.2†	13.9±2.0	13.8±2.2
Peak VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$) (n=7 M, 5 F)	56.4±1.2	59.8±0.7	47.9±1.3	50.8±1.6
BMD - L1-L4 ($g \cdot cm^2$) (n=4 F)	Not Tested at this time		1.31±0.09	1.28±0.06
BMD - F. Neck ($g \cdot cm^2$) (n=4 F)	Not Tested at this time		1.17±0.02	1.15±0.02*

† p=0.024, * p=0.019 vs. Pre-Season

During the course of the 1997 fire season, TEE was determined using the doubly labeled water technique in a select number of subjects (n=5). At the time this report was completed, only one subjects samples have been completely analyzed using isotope ratio mass spectroscopy. This approach was warranted as TEE has not been previously measured in this population. Therefore, it was our objective to obtain data on one subject in full to more specifically address future sample analyses. This subject population is also a challenge because total body water turnover was expected to be elevated above well above normal (due to heat exposure and daily ingested water volumes). Therefore, the initial dose of isotope was increased to account for the anticipated rapid isotopic decay. We were also interested in the possibility of using a shorter collection period (i.e. 3 day) as this is more often representative of arduous wildland fire suppression.

Table 19 represents the calculated isotopic decay constants for both the entire five days of collection and also the initial three days of collection. Based on this comparison, there are noticeable differences between the average daily rates of energy expenditure. However, as is common with most wildland fire suppression incidents, the majority of the strenuous work (self reported) was completed during the initial three days of operations. Regardless, these values for TEE are higher than anticipated and represent a challenge to the maintenance of energy balance. At this time, dietary assessments have not been completed to determine whether the average daily intake was hyper or hypocaloric.

The objective of obtaining precise measures of daily and TEE is to determine whether periods of elevated energy expenditure are associated with increased rates

of bone resorption or breakdown and whether this response is gender specific. At this time, however, post season sampling is not complete. Therefore, the assay selected as the marker of bone resorption (Osteomark- NTx) will not be performed on seasonal samples until post season samples are collected. Post season collections are expected to be complete at the early part of November, 1997.

Table 19. Total energy expenditure (TEE) for one male subject during a five day collection period.

Subject (DH) **Variable**	
Five Day Analysis	
kO (Oxygen-18 decay constant)	-0.1612
kH (Deuterium decay constant)	-0.1233
CO2 Production (mol/day)	46.36
kcal/day (base on 5 day collection)	**5,911**
Three Day Analysis	
kO (Oxygen-18 decay constant)	-0.1581
kH (Deuterium decay constant)	-0.116
CO2 Production (mol/day)	51.42
kcal/day (base on 3 day collection)	**6,556**

Conclusions

At this time it is difficult to formulate conclusions based on the completion of one subjects TEE data. Regardless, these values for TEE measured during wildland fire suppression are somewhat higher than values previously measured during military operations (see Table 20). Therefore, this subject population may serve as

an ideal model to determine the effects of arduous occupational exposure on bone health during un-simulated field operations.

Table 20. Measures of TEE using the doubly labeled water method during military operations.

Investigation	Activity	kcal/day
Hoyt et al. (1991)	Mod. cold/altitude, heavy ex.	4919±190
Jones et al. (1993)	Ext. cold, moderate ex.	4317±293
Hoyt et al. (1994)	Mod. cold/altitude, heavy ex.	4639±231

Problem areas associated with fire season data collection

The nature of the doubly labeled water technique is straightforward and relatively simple in terms of the dose and sample collection procedure. However, it was very difficult to precisely determine when the dose should be administered. The 1997 fire season in the Northwest was extremely slow with limited activity. In late July, there was an incident near Lincoln, MT and we were deployed with the two Hot Shot crews and our subjects. Upon arrival, all weather reports and projections suggested that the small 50 acre fire would spread and remain active for no less than five days. We used this as our decision making instrument and committed a dose of doubly labeled water to one male and one female subject. By day three, the weather had changed dramatically and fire suppression was no longer necessary. However, the day we returned from the incident in Lincoln, MT, both Hot Shot crews were deployed to the Hopper incident near Fillmore, CA (a 25,000 acre fire). At this fire, we were able to dose two females and one additional male subject. As the dose requirements are higher compared to average individuals, it is very costly to use this technique. During this season, we were somewhat

conservative because TEE has not been measured in a population with extremely high rates of water turnover (due to heat exposure and ingested water volumes). Therefore, with this seasons data, we hope to extend our sample size considerably for next fire season.

The other difficulties surrounding seasons data collection was the realization that these subjects are subjected to "real world" situations which do not slow down or stop for the sake of data collection. For this reason, the sample size and the subject availability is relatively small. It was also difficult to collect samples in the fire camps at times because subjects sometimes spent the night in a remote camp on the fire. However, because the peptides (NTx assay) and water within the urine samples are stable, self collection in the remote camps was appropriate. When samples were collected away from the main fire camp location, they were always returned to us within 10 hours following collection.

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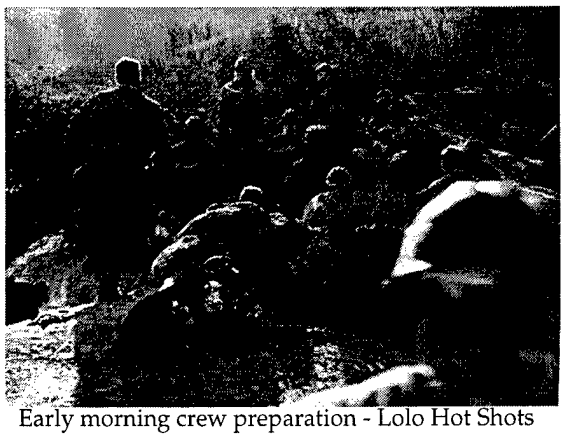
Appendicies

Appendix I - Photographs from field data collection

Hopper Incident, Fillmore, CA (August, 1997)
Early morning (0500) on the fireline - urine collection for measures of total energy expenditure. Brent Ruby, Ph.D. at right (Principal investigator), Ted Zderic at left (research assistant)

Ted Zderic removing the heart rate monitor from a subject after a days work on the fireline.



Early morning crew preparation - Lolo Hot Shots

Appendix II Original Statement of Work

The following statement of work and specific tasks will allow for the most efficient use of laboratory and field measurements in accordance with the anticipated fire seasons (Spring/Summer of 1997 and 1998). Prior to funding availability, some tasks will be performed to prepare for the anticipated date associated with data collection (noted as negative numbers on the schedule below).

Task 1: Months -4 to -1 (May - Aug): Devise a working schedule for fire season 1997 collection period. Perform pilot work "on location" during the 1996 fire season and develop logistics for planned measurements. Obtain final institutional human subjects approval for planned investigation(s).

Modification: NA - funds were not available until September 23, 1996 therefore no data was collected during this time. At this time, the necessary approvals for the use of USFS employees as subjects and for data collection during wildland fire supression were being discussed.

Task 2: Months 1 to 2 (Sep-Oct): Recruit subjects and collect serial blood samples (females) for the determination of menstrual/reproductive hormones in preparation for laboratory testing procedures.

Modification: Because of the use of Interagency Hot Shot crew members, this task was also not possible. Crews are not assembled until late May/early June and are disassembled by October. However, additional time was necessary for the final approval process for the use of Interagency Hot Shots.

Task 3: Months 3 to 4 (Nov-Dec): Analyze all baseline blood samples. Schedule subjects for laboratory testing in accordance with menstrual cycle phase and gender. Initiate gender comparison study using controlled laboratory testing procedures.

Modification: At this time, no samples had been obtained and no subjects had been tested for reasons listed above. However, the gender and menstrual comparison study component was initiated at this time.

Task 4: Months 5 to 10 (Jan-May): Complete gender comparison laboratory tests and analyze results. Based on controlled data collection evaluate and plan final testing procedures to be used during the 1997 fire season field collection studies. Collect pre-season baseline blood samples during months 9 and 10 on female subjects in

preparation of fire season testing. Collect baseline measures of total bone mineral content using mobile DEXA system. Obtain pre/early season descriptive laboratory measures (pulmonary function, body composition, aerobic capacity, DIT).

Modification: The data collection for the gender/menstrual comparison study was completed during this time. Several Interagency Hot Shot crews were contacted for the subject selection process. Meetings were held with all perspective Hot Shot crews and subjects from two crews were selected for the upcoming 1997 fire season.

Task 5: Months 10 to 15 (May-Sep): Fire season field (extended operations) collection period. Obtain seasonal measures of TEE and other measures surrounding doubly labeled water techniques. Obtain weekly samples for reproductive hormones and bone resorption. Obtain self-reported daily task and injury log.

Modification: Data collection proceeded as planned. In addition, samples from the gender/menstrual comparison study were being analyzed at this time.

Task 6: Months 16 to 17 (Oct-Nov): Obtain post-season measures (bone mineral content, body composition, aerobic capacity, job specific performance testing).

Modification: Post season measures have been initiated at this time. However at the time of this annual report, not all of the 1997 seasonal samples have been analyzed. **Report ending October 22, 1998.**

Appendix III

Approval for the use of USFS employees and research activity during wildfire supression

- Revised and secured approvals of the memorandum of understanding between the University of Montana Human Performance Laboratory and the Forest Service Technology and Development Center (MTDC).

- Reviewed study plan with and secured approvals from Forest Service (FS):
  MTDC Fire and Aviation Management (F&AM) Program Leader (D.Mangan)
  FS Regional Office Director of F&AM (J. Williams)
  FS Washington Office F&AM Staff (D. Aldrich)
  FS Director of F&AM (M.J. Lavin)

- Secured letters of authorization from MTDC and the FS Director of F&AM.

- Met w FS Region 1 Hotshot Crew leaders to describe study and secure cooperation.

- Met with FS Region Smoke Jumper leaders to describe study and secure cooperation.

- Met with Lolo and Bitterroot Hot Shot crew leaders; then met with crew members to discuss details.

- Met with Smoke Jumpers to enlist subjects and discuss details of data collection.

- Investigators participated in pre-season fire training and secured "red card" certification required by all firefighters and those engaged in fire camp activities.

- Prepared a job hazard analysis to cover anticipated activities of investigators during field data collection.

- Met with FS Aerial Fire Depot dispatchers to discuss logistics and notification of crew mobilization.

- Similar meetings with Montana State and Bitterroot National Forest dispatchers to insure timely notification of crew mobilization.

- Outfitted investigators with NFPA approved firefighter uniforms and fire shelters.

Appendix IV - Proposed presentations associated with project - 1998

National ACSM - Orlando, FL - 1998

Gender difference in glucose kinetics during exercise. Ruby BC, AR Coggan, T Zderic, and BJ Sharkey.

Effects of the menstrual cycle on glucose kinetics during exercise. Zderic T, AR Coggan, BJ Sharkey, and BC Ruby.

Efficacy of field heart rate measures for the prediction of energy expenditure in wildland firefighters. Burks C, S Tysk, BJ Sharkey, and BC Ruby.

Northwest Regional ACSM meeting - Whitefish, MT - 1998

Use of field measures for heart rate to estimate occupational specific energy expenditure in wildland firefighters. Runge N, C Burks, S Tysk, BJ Sharkey, and BC Ruby.

Seasonal changes in bone density relative to total energy balance and gender in wildland firefighters. Vemich E, BJ Sharkey, and BC Ruby.

Gender specific responses to exercise intensities relative to the lactate threshold. Burks C, T Zderic, BJ Sharkey, and BC Ruby.

Texas Regional ACSM meeting - Arlington, TX - 1998

Effects of the menstrual cycle on glucose kinetics during exercise. Zderic T, AR Coggan, BJ Sharkey, and BC Ruby.

Appendix V - Personnel receiving salaries from project

Principal Investigator	Brent C. Ruby, Ph.D.	9/96 - present
Co Investigator	Brian J. Sharkey, Ph.D.	9/96 - present
Research Assistants	Theodore Zderic (Laboratory and Field Assistant)	9/96 - 5/96 & 6/96 - 8/96
	Shelly Johnson (Nutrition and Field Assistant)	6/96 - 8/96
	Jake Swan (Laboratory Asssistant)	6/96 (approx. 10 hrs.)
	Catherine Burks (Laboratory Assistant)	9/97 - present
	Sonja Tysk (Laboratory Assistant)	9/97 - present