PERCEIVED EXERTION AND WORK CAPACITY DURING THE MENSTRUAL CYCLE

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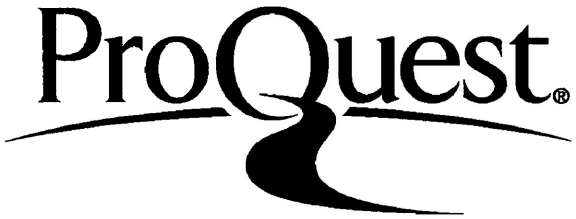

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ABSTRACT

The purpose of this study was to identify and confirm biochemically three phases of the menstrual cycle in 14 well conditioned athletes and then to examine the effects of the various phases on work capacity, perceived exertion and lactic acid accumulation following individualized work tasks. Radioimmunoassays of plasma estradiol and progesterone were used to confirm the midluteal and mid-follicular phases of the cycle. Subjects performed a 12 minute sub-maximal work task consisting of 3 minutes at 60%, 3 minutes at 70% and 6 minutes at 80% of their measured maximum work capacity. Perceived exertion was recorded every 2 minutes using the Borg 15 Point Scale. After a 15 minute rest the subjects were asked to run as long as they could at a work rate corresponding to 100% of their maximum work capacity. Blood samples were drawn for lactic acid assays prior to the start of the initial run, 2 minutes after the sub-maximal run and 5 minutes after the run to exhaustion.

The phase of cycle had no influence on perceived exertion or on lactic acid accumulation. Maximum run times to exhaustion increased significantly ($p < .05$) in the mid-follicular and midluteal phases when compared to day 1. The average increases were 17.9% and 40.2% respectively. These findings indicate that work capacity is maximal in the midluteal phase.

TABLE OF CONTENTS

		Page
ACKNOWLEDGEMENTS		i
ABSTRACT		ii
LIST OF FIGURES		v
LIST OF TABLES		vi
CHAPTER I	INTRODUCTION	1
	Statement of the Problem	1
	Significance of the Study	1
	Delimitations	3
	Limitations	3
	Definitions	4
CHAPTER II	REVIEW OF LITERATURE	6
CHAPTER III	METHODS AND PROCEDURES	14
	Research Design	14
	Subjects	14
	Establishing Work Intensities	15
	Perceived Exertion (RPE)	16
	Basal Body Temperature	18
	Progesterone, Estradiol and Lactic Acid Measures	18
	Cycle Testing	19
	Data Analysis	20
CHAPTER IV	EXPERIMENTAL FINDINGS	22
	Description of Subjects	22
	Hormonal Confirmation of Phase of Cycle	22
	Determination of Maximum Work Capacity and Ratings of Perceived Exertion	26
	Maximal Run Time (MRT)	26
CHAPTER V	DISCUSSION	37
CHAPTER VI	SUMMARY AND RECOMMENDATIONS	44
	Summary	44
	Recommendations	46

Page

REFERENCES 47

APPENDICES

A. Questionaire on Menstrual Cycle, client history questionnaire 51

B. Borg's 15 Point Scale for ratings of perceived exertion 55

C. Immuno-ESTRADIOL iodine-125 kit, PANTEX 047 56

D. No Extraction Progesterone 63

E. Lactic Acid Assay 68

F. Perceived Exertion Values 72

G. Maximum run time, lactic acid, estradiol and progesterone values 73

LIST OF FIGURES

Figure		Page
1.	Variation of plasma progesterone over the course of the cycle	10
2.	Variation of 17 estradiol during the course of the menstrual cycle	11
3a.	Run times to voluntary exhaustion at 100% work capacity subjects 1-5	30
3b.	Run times to voluntary exhaustion at 100% work capacity subjects 6-10	31
3c.	Run times to voluntary exhaustion at 100% work capacity subjects 11-15	32

LIST OF TABLES

Table		Page
1.	Modified Balke Treadmill Test	17
2a.	Estradiol and progesterone plasma levels to confirm phase of menstrual cycle subjects 1-8	24
2b.	Estradiol and progesterone plasma levels to confirm phase of menstrual cycle subjects 9-15	25
3.	Perceived exertion at minutes into submaximal run at day 1, midfollicular and midluteal phases	27
4.	Personal data, max MET, and submaximal work load assignments for measures of RPE	28 & 29
5.	Maximal run time and phase of cycle	33
6.	Lactic acid levels	35
7.	One way analysis of variance of RPE, MRT, lactic acid, estradiol and progesterone with phase of menstrual cycle	36

CHAPTER I

INTRODUCTION

Statement of the Problem

The purpose of this study was to identify and confirm biochemically three phases of the menstrual cycle in well-conditioned athletes and then to examine the effect of the various phases on work capacity, perceived exertion and lactic acid accumulation following assigned work tasks.

Significance of the Study

Female athletes have long held that they were at a disadvantage whenever required to compete while menstruating. A corollary to this has been the belief that they fared better in competitions scheduled at a time when they were not menstruating. Many studies have been carried out to test the relationship between the phases of the menstrual cycle and maximal physical performance. Some studies have concluded that menstruation adversely affected performance in activities such as gymnastics and tennis, while other studies have found menstruation to have little or no effect on performance in swimming or cycling (Doolittle and Engebretsen, 1972; Garlick and Bernaver, 1968; Scott and Tuttle, 1932). Still other studies were unable to find any relationship between the phase of cycle and achievement (Erdelyi, 1962; Pierson and Lockhart, 1963). Some of the confusing or contradicting conclusions may have been caused

by the inaccurate calendar method of identifying the phases of the cycle. Errors in assigning phases may have produced a masking effect on bonafide cycle-related performance variations (Doolittle and Engebretsen, 1972; Gamberale, Strindberg and Wahlberg, 1975; Wearing, et al 1972).

In recent years, it has become possible to confirm biochemically the midfollicular and midluteal phases. Radioimmunoassays of plasma levels of 17-B estradiol (E-2) and progestrone (P) allow the measurement of levels typical of these phases (Henry, 1970; Ros et al 1970). Both E-2 and P are present in low levels during menstruation. By the time the midpoint of the follicular phase has been reached, the E-2 level, but not the P level has become elevated. At the middle of the luteal phase, levels of both E-2 and P are very elevated.

The phases under observation were day 1 of menses, the midfollicular phase (day 7 $\pm$ 1) and the midluteal phase (day 21 $\pm$ 1) of the menstrual cycle. The maximal work capacity was determined for each subject and the perception of effort to perform at 80% of maximal work capacity was recorded during each phase of her cycle.

Findings from this study could help predict whether an athlete's performance in a strenuous event might be better if the event took place at a time when she was in one identified phase rather than in some other phase of her menstrual cycle. Positive findings might suggest that the athlete's menstrual cycle be interrupted or influenced by

use of oral contraceptives, so that at a time of a major competition the athlete would be in a phase of her cycle when her capacity to perform was optimal.

Delimitations

Fourteen well conditioned female athletes ranging in age from 18 to 34 with 'normal' regular cycles were tested over two mentrual cycles. No subject was taking oral contraceptives. (Data are included on one additional subject who was on oral contraceptives, but her test findings were kept separate and are indicated by an asterisk.) Diurnal variations in hormones were avoided by not varying the time of conducting the tests for each subject. Subjects were instructed not to eat for 3 hours before commencing a test session. All tests were carried out between 1700 hours and 2000 hours for each subject. To eliminate any training or practise effects on performance in a particular cycle phase, 5 subjects started the testing program at day 1 of menses, 5 others started at the midfollicular phase, and 4 started at the midluteal phase of their cycle.

Limitations

The study was conducted at the Paul Schwaan Fitness Center at the University of Regina in the period from November 15, 1982 to March 31, 1983.

Definitions

Balke Treadmill Test is a measure of work capacity where subjects perform at a constant speed, while the incline of the treadmill is raised 2.5 degrees every 2 minutes, until the subject is unable to continue.

Endurance Time refers to the number of seconds that a subject runs while performing at 100% of her work capacity.

17-B Estradiol (E-2) is the hormone principally responsible for the development of female secondary sex characteristics. Blood levels of E-2 reach a peak just before ovulation, decline at the time of ovulation, and reach a second peak a few days after ovulation.

Menses or menstruation is the periodic discharge from the vagina of blood and tissue from a non-pregnant uterus.

Menstrual Cycle is the period from the onset of menses (day 1) to the day before the start of the next menses. The length of the cycle in the 14 subjects used varied from 26 to 31 days, with a mean $\pm$ standard error of 28.2 $\pm$ 2 days.

Met is the metabolic equivalent of the basal metabolic rate equal to 3.5 milliliters of oxygen per kilogram of body weight per minute. (1 Met = 3.5 ml/kg/min.)

Midfollicular Phase of the menstrual cycle occurs 6 to 9 days after the start of menstruation. It is the midperiod of follicle growth preparatory to ovulation. It is characterized by very low levels of P and slightly elevated levels of E-2.

Midluteal Phase of the menstrual cycle occurs 20 to 23 days after the start of menstruation. It is characterized by high levels of E-2 and high levels of P in the circulating blood.

Perceived Exertion (RPE) is a scaled estimate of how difficult a task is perceived to be by the subject.

Progesterone (P) is the female hormone secreted by the corpus luteum commencing with ovulation. Its function is to prepare the uterus to receive a fertilized ovum.

Lactic Acid (LA) is a metabolite produced in muscle tissue during anaerobic metabolism.

Maximum Work Capacity (Wmax) is the highest work load successfully maintained for 1 1/2 minutes using the Balke Treadmill Test.

CHAPTER II

REVIEW OF LITERATURE

Physiological reasons have long been sought to explain the many reports of poor athletic performances by females just prior to or during menses (Erdelyi, 1964; Zaheieva, 1965; and Webb, Millian and Stolz, 1979).

Moore (1923) made daily measurements of the strength of eight muscle groups over many menstrual cycles. He concluded that during menstruation, the strength of each group of muscles was less than the strength in the intermenstrual periods. This finding was confirmed by studies of Howland (1936) and Keenan (1958). However, Carson (1966) found that only the strength of one muscle group was adversely affected by menstruation, with other muscle groups being unaffected. Wearing et al, (1972) found that the best achievement in three tests of muscle strength were generally to be found about seven days prior to menstruation. Other studies found no changes in muscle strength that could be dependably related to the phase of the menstrual cycle (Auster, 1944; Rockwell, 1962; Petrofsky, 1976; Higgs and Robertson, 1981).

Other investigators have examined the relationship between reaction times or movement times and the phase of the menstrual cycle. Dalton (1960) found that individuals had slower reaction times when menstruating. Gamberale (1975) and Hunter, et al (1979) found decision reaction-times were longer during menses. Other investigators found

that there were no changes in simple reaction time or movement time that could be associated with a particular phase of the mentrual cycle (Pierson and Lockhart, 1963; Koppel et al 1969).

Possible variations in maximal cardiorespiratory capacity with a phase of the menstrual cycle have also been examined. Doolittle (1972) in a study using four performance tests each requiring near maximal cardiorespiratory output, found no measureable differences in performance over the course of the menstrual cycle. The findings of Phillips (1973) and Fox, et al (1969) also found no measureable differences when they measured metabolic and cardiorespiratory responses to exercise. Allsen, Parsons and Bryee (1977) investigated maximal oxygen uptakes at each of four phases of the menstrual cycle, but found no significant differences. Heart rates and oxygen uptake at submaximal as well as at maximal work loads were found by Doolittle (1972) and Stephenson et al (1982) to be unrelated to phase of the menstrual cycle. Other investigators were unable to find a correlation between any phase of the menstrual cycle and the fluid volumes of hematocrit or the level of hemoglobin during exercise (Dintenfass, 1966; Garlich and Bernauer, 1968; and Albohm, 1976).

Psychological factors that might explain poor performance during menstruation were examined by a number of investigators. Pierson and Lockhart (1963) suggested that the poorer performances and higher rates of accidents among

menstruating women were due to discomfort and external distractions. They found a linear relationship existed between heart rate, oxygen consumption, and a subject's perception of effort (Borg, 1973; Morgan, 1973; Skinner, Hustler, Bergsteinova and Buskirk, 1973; Hage, 1981). Gamberale (1975) found that female athletes who suffered from dysmenorrhea perceived work to be more difficult immediately prior to or during the flow phase. This increase in perception of difficulty was recorded at work loads as low as 40% of the subject's work capacity. Petrofsky et al (1976) observed that some women using oral contraceptives displayed no change in performance scores or perceived exertion over the course of a menstrual cycle when working at submaximal or maximal work loads. Other investigators found a distinct increase in perceived exertion of subjects performing at maximal capacity just prior to and on day 1 of menses, but found no increase in perceived exertion when performing submaximal work tasks (Gamberale et al, 1975; Higgs and Robertson, 1981; Stephenson et al, 1982). The expression of a subject's perception of exertion was refined to a 15 point scale by Borg (1982) to allow better standardization of this value (now referred to as RPE - achronym for ratings of perceived exertion).

The inconsistent physiological explanations for poor performances during menses might have resulted from inaccurate recognition of the phase of cycle when the measurements were made (Doolittle et al, 1975). Confirmation of the

phase of cycle became possible with the advent of radioimmunoassays and other very sensitive methods of measuring female hormones. Fluctuations that occur in the circulating levels of 17-B estradiol and progesterone over the course of a menstrual cycle are shown in Figures 1 and 2. Jurkowski, Jonas, Walker, Vounglai and Sutton (1978) used such measures to confirm the phase of cycle and went on to assess the effect of exercise on hormone levels, cardiovascular output, and respiratory response at each phase of the cycle. Subjects exercised in a progressive manner for 20 minutes at both 30-35% and 60-66% of maximum power output. This was followed immediately by a 85-95% effort to exhaustion. No differences in the maximal work load achieved, heart rate, or ventilation, could be associated either with the low levels of estradiol and progesterone in the follicular phase, or with the high levels of both hormones during the luteal phase. The circulating levels of estradiol and progesterone were found to be dependent upon both the intensity of the exercise and upon the phase of the menstrual cycle. Estradiol levels increased during exercise in both phases, the greatest increase occurring at maximal exercise intensities. Progesterone followed this pattern during the luteal phase, but during the follicular phase levels remained low regardless of exercise intensity. Bonen and Belcastro (1978) found similar responses of these hormones to exercise during the follicular and luteal phase, but found that the estradiol increased only marginally with exercise during menses.

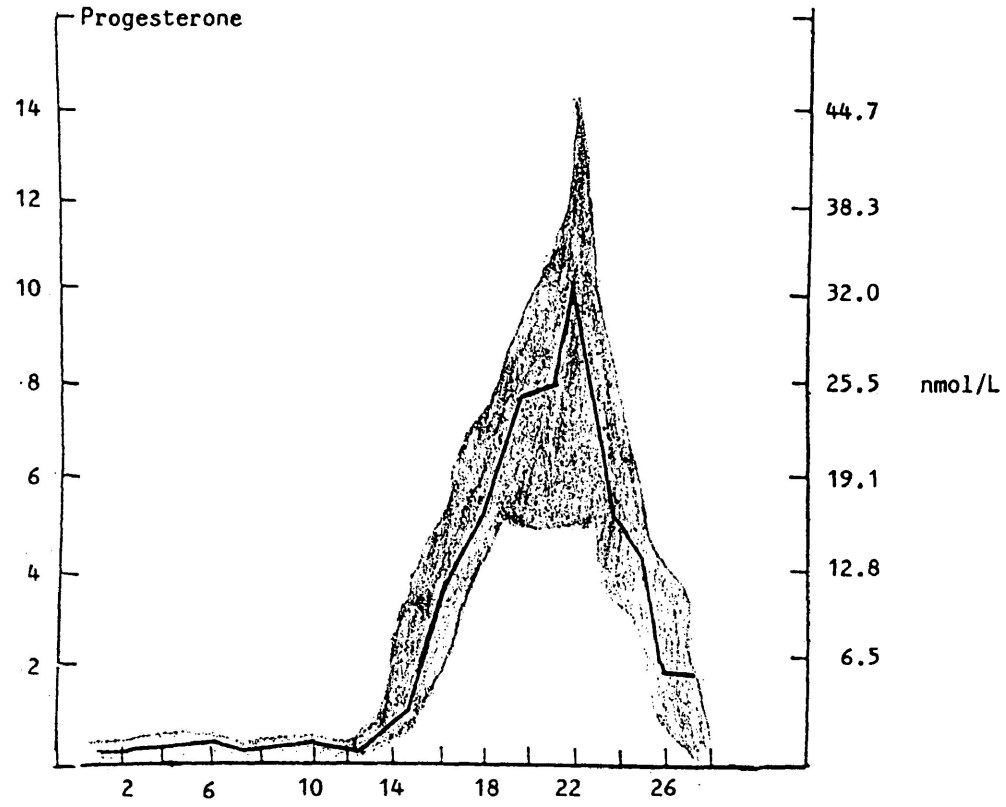

Figure 1. Variation of plasma progesterone over the course of 16 presumptive ovulatory cycles, synchronized around the day of the LH midcycle peak. Shaded area represents 95% confidence limits around means (bold line) (Ross, G.T., Cargille, C.M., Lipsatt, M.B., Rayford, P.L., Marshall, J.R., Strait, C.A., and Rodbard, D. Pituitary and Gonadal Hormones in Women. Recent Progress in Hormone Research 26, 1970).

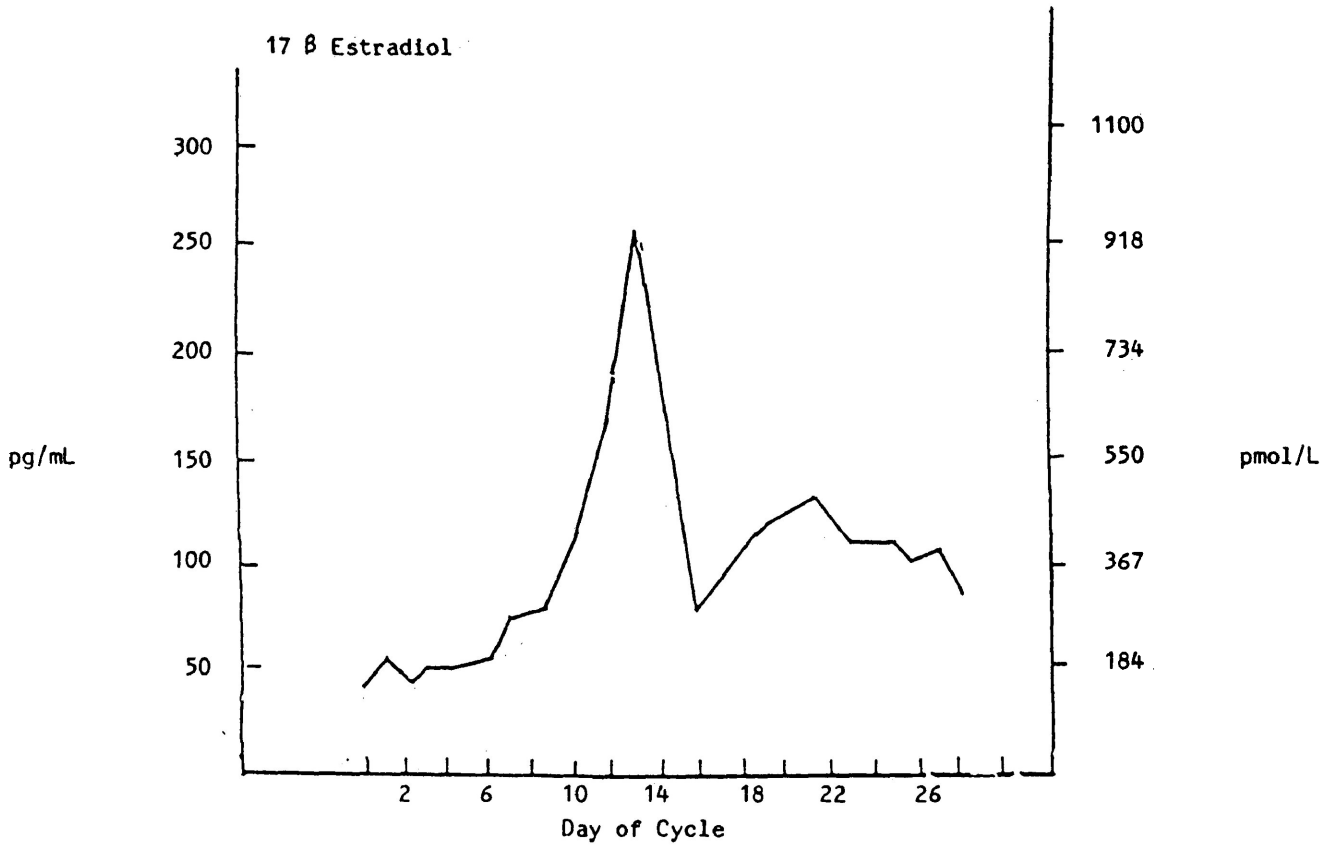

Figure 2. Evolution of 17 β estradiol during the course of the menstrual cycle. The cycle is centered on the mid LH peak. (Modified from Franchimont, P., Valcke, J.C., and Lambotte, R. Evaluation of Endocrine Function. In J.B. Henry (Editor). Clinical Diagnosis and Management by Laboratory Methods, W.B. Saunders Co., 1979.)

Jurkowski et al (1978) found that the time to exhaustion at 90% of maximum work load was significantly longer during the luteal phase than in the follicular phase. It had been suggested that, since the progesterone and estradiol are at their highest levels during the luteal phase, they might alter performance capacity through effects on substrate metabolism (Bonen, Ling, MacIntyre, Neil, McGrail and Balcastro, 1979). It would follow that the low levels of both hormones during menses might account for the performance decrements experienced by many females at time of menses. A further study by Jurkowski (1982) found that plasma lactate was higher in response to heavy and exhaustive exercise during the follicular phase than in the luteal phase. This higher plasma lactate could be due either to higher rate of production, or due to slower removal by liver or kidney under altered hormone level.

One theory is that high circulating hormone levels of progesterone and estradiol in the mid-luteal phase may facilitate an augmented response during exercise, which somehow at high exercise intensities results in a more rapid removal of lactic acid and increased work-time to exhaustion (Higgs, Note 1). Another theory is that the high levels of ovarian hormones in the blood during exercise facilitate improved performance by either (a) enhancing oxidation of fatty acids thereby delaying glycolytic metabolism, or (b) improving utilization of oxygen in the muscle (Jurkowski, 1982).

In conclusion, there appear to have been few studies in the literature that have dealt with the influence of the various phases of the menstrual cycle on athletic performance when the phases of the menstrual cycle were biochemically confirmed. There is a need for studies which biochemically confirm the phases to ensure accurate assignment of each menstrual phase when tests are performed. With the use of individualized work tasks, it might be possible for a performance high point to be more clearly identified with some phase of the menstrual cycle.

CHAPTER III

METHODS AND PROCEDURES

Research Design

An individualized subject design employing repeated measures at three recognizable phases of the menstrual cycle was used to investigate possible related performance changes. Performances were compared on the first day of menstruation, in the midfollicular phase, and in the midluteal phase. The performance parameters investigated were ratings of perceived exertion during a twelve minute submaximal run, and a timed run-to-exhaustion at each individual's maximal work capacity. Blood for lactic acid assays were drawn 2 minutes after completion of the submaximal run and again 5 minutes after completion of the run-to-exhaustion. Assessments and measurements were made over 2 menstrual cycles so that the consistency or variability of each individual's performance could be recognized.

The Subjects

Fourteen healthy, physically well-conditioned female athletes ranging in age from 18 to 33 years served as subjects. (Data are included on a fifteenth subject who was on oral contraceptives. Her data was kept separate and are indicated by an asterisk.) The predicted $M\dot{V}O_2$ for the subjects ranged from 39.2 ml/kg/min to 53.6 ml/kg/min with the average being 46.0 ml/kg/min. All subjects were

questioned regarding medical and menstrual history (Appendix A). All were deemed to be healthy and to have cycles falling within normal limits.

The study was conducted over a five month period, November 1982 through March 1983. During this time subjects agreed to maintain a constant activity level. All testing was conducted at the Dr. Paul Schwaan Fitness Centre at the University of Regina. Each subject was tested at the same time of day throughout the study, and all testing sessions were completed within 24 hours of the target day. The order of introduction to testing was varied so that five subjects had their first test on day 1 of menstruation, four were first tested in their midfollicular phase, and five were first tested in the midluteal phase. The subject on oral contraceptives entered the study on day 1 of menstruation.

Establishing Work Intensities

Prior to entrance into the study, individual maximal work capacities were assessed during days 7-9 of the menstrual cycles, using the modified Balke Treadmill Test (Liss, 1980).

Work capacities were measured on a Quinton power driven treadmill. Individuals selected a starting speed with which they were comfortable. The speed was held constant and the inclination was increased 2.5 degrees every 2 minutes until the subject was no longer able to continue. All subjects were made aware of the importance of giving an all out effort.

An individual was considered to have successfully completed a given work load if she had maintained that work load for a minimum of one and a half minutes. The highest work load successfully maintained was regarded as the individual's maximal work load as measured in METS (Table 1). The MET value indicated by the maximal work load was used to determine work levels for the 12 minute submaximal runs.

Perceived Exertion (RPE)

Perceived exertion was estimated using the Borg 15 Point Scale (Appendix B). The instrument was constructed using numbers ranging from 6 to 20, with work intensity categories assigned to odd numbers. Several studies agree that RPE values increase linearly with intensity of work (Borg and Linderholm, 1967; Gamberale, 1972; Borg, 1982; Morgan, 1973).

Perceived exertion was defined for the subjects as "how hard or difficult the exercise feels to you". They were informed that a score of 6 represented a comfortable sitting or standing value and a score of 20 represented complete exhaustion or inability to continue work. The scale was placed in front of the treadmill during determination of work capacity, and subjects were asked to constantly assign values to the work they were doing.

Table 1

Relative Energy Expenditure in METS
for Graded Exercise

(Modified Balke Treadmill Test)

Grade %	Duration	Expenditure in METS*				
		Speed				
		5.0mph	5.5mph	6.0mph	6.5mph	7.0mph
0	2 min	8.6	9.3	10.0	10.8	11.5
2.5	2 min	10.0	10.6	11.4	12.0	12.7
5.0	2 min	11.4	12.0	12.7	13.3	14.0
7.5	2 min	12.6	13.2	13.9	14.6	15.3
10	2 min	13.9	14.5	15.2	15.8	16.5
12.5	2 min	15.4	16.0	16.5	17.2	17.8

MET Value

*MET Calculation 1 MET = 3.5 ml/kilogram/min (of oxygen cost)

Basal Body Temperature

All subjects were supplied with a basal body thermometer and agreed to record basal body temperature each morning before getting out of bed. A sustained increase in basal body temperature of at least 0.3 degrees Celsius was used as an indicator that ovulation was taking place. Testing for the midluteal phase was conducted 7 days following the temperature rise. Cycle phase was confirmed through analysis of the hormones progesterone and estradiol in venous blood.

Progesterone, Estradiol and Lactic Acid Measures

Venous blood samples were drawn by a Registered Medical Laboratory Technologist. Three samples were drawn from each subject at each session. The first sample was collected while the subject was at rest prior to the start of each test session, the second sample was drawn 2 minutes following the submaximal work task, the third specimen was drawn 5 minutes following the work task to exhaustion.

Blood samples were drawn into 5 ml heparinized vacuum tubes. To prevent clotting, the tubes containing the whole blood were mixed by inverting them 10-15 times in a one minute period. Exactly one millilitre of the heparinized whole blood was then removed with an Eppendorf pipette and injected into a clean 16 mm x 125 mm glass tube containing 2 ml of ice cold 0.6M perchloric acid. The tube was stoppered with a paraffin waxed cork, mixed well by shaking, and placed in an ice water bath for a minimum of 5 minutes. At

that time the tubes, containing the 1 ml of whole blood in 2.0 ml of perchloric acid, were centrifuged in an I.E.C. clinical angle head centrifuge. The tubes were spun at 2000 r.p.m. for a minimum of 5 minutes. The supernates were transferred to separate clean glass tubes and placed in a refrigerator for analysis of lactic acid within 24 hours, or frozen at -20 degrees Celsius for delayed batch analysis. The plasma supernate was used for analysis of progesterone and estradiol levels.

All analyses of the hormones progesterone and estradiol, and blood lactate levels were performed under contract by the Saskatchewan Provincial Health Laboratories. Estradiol was assayed by radiommunoassay using Pantex immuno-Estradiol 125-I kits (Appendix C). Progesterone was analyzed by a No-Extraction radioimmunoassay with reagents purchased from Diagnostic Products Corporation (Appendix D). Lactic acid was assayed on an Abbott 200 analyzer using procedures and reagents detailed by Sigma (Appendix E). Controls and blanks on all assays were monitored carefully in compliance with standard techniques.

Cycle Testing

Upon reporting to the laboratory, subjects completed a daily living questionnaire, were weighed, and had the resting blood sample drawn. Subjects then performed a continuous 12 minutes submaximal work task involving 3 minutes at each of 60% and 70% of their maximal work capacity, and 6 minutes

at 80% of their maximal work capacity. Subjects were reminded to give ratings of perceived exertion that reflected how difficult the task felt to them at that moment. Ratings of exertion were recorded at the end of every 2 minutes (Appendix F). The 12 minute work task was followed by a fifteen minute rest period during which time the subject remained seated. The second blood sample was drawn 2 minutes into the rest period.

The timed run to voluntary exhaustion at 100% of the individuals maximum work capacity followed the rest period. Subjects were encouraged to work as long as possible, and it was stressed that every second was important. Time into the work task was announced every 30 seconds, and gentle verbal encouragement was given when subjects started to struggle.

The third blood sample was collected five minutes after the termination of this run. Subjects remained in the laboratory until fully recovered.

Data Analysis

Graphs were prepared to illustrate any trends in the various parameters in each individual over the period of 2 cycles. Individual data have been presented in tabular as well as in graphic form. Described are ratings of perceived exertion, maximal run time, and pre- and post work levels of blood lactic acid at each of the three phases of the menstrual cycle. Within subjects average values have been used in a one-way analysis of variance (ANOVA for the

repeated measures of mean RPE, mean lactic acid and mean all out performance time). Any significant differences revealed by the ANOVA have been followed up by post-hoc comparisons.

CHAPTER IV

EXPERIMENTAL FINDINGS

Description of Subjects

Subjects ranged in age from 18 to 33, and all were deemed to have cycles falling within normal limits. The predicted maximum oxygen consuption ranged from 39.2 ml/kg/min to 53 ml/kg/min, with an average for the group of 46.0 ml/kg/min. All subjects were actively involved in some form of athletic activity at least 3 times per week. Personal data for each subject can be seen in Table 4.

Hormonal Confirmation of Phase of Cycle

Measures of the resting levels of plasma progesterone and plasma estradiol were utilized to confirm the stage of each subjects menstrual cycle when her performance was being assessed. Some authors claim hormone levels may be affected by exercise (Bonen and Belcastro, 1978), therefore, blood for hormone analysis was collected at rest prior to commencement of exercise.

Lowest values of progesterone are observed during the follicular (preovulatory) phase of the normal menstrual cycle. The level increases sharply after the mid-cycle peak of luteinizing hormone, becoming very high in the luteal phase at or around 21 days of the cycle. Levels fall off sharply towards base-line by the onset of menstruation. The

level of progesterone does not invariably return to base-line values before the onset of menstruation, so low levels of progesterone found at day 1 of menses may not be as low as levels found at day 7 (Yen et al 1970).

Estradiol levels follow a biphasic pattern over the menstrual cycle. From a low of less than 200 pmol/L at day 1, the level slowly increased until about day 7 when levels up to 400 pmol/L may be found. The estradiol levels then rose sharply to reach a maximum as high as 1200 pmol/L at mid-cycle, sometimes designated as the 'ovulatory peak'. Levels then fell off for a few days before they increased to a second but lower peak in the luteal or "premenstrual phase". This second peak developed at about 21 days (midluteal phase) of the normal menstrual cycle (Speroff and Vande Wiele, 1971).

Reviews of simultaneous measures of estradiol (E-2) and progesterone (P) levels allowed confirmation of the phases of the cycle for all participants at day 1, midfollicular, and midluteal. For example: on day 1, both E-2 and P are low; at midfollicular phase the E-2 is low but higher than on day 1 and P is low, possibly lower than on day 1; at midluteal phase both E_2 and P are very elevated.

Actual levels used to discriminate are shown in Tables 2a and 2b.

Table 2a

Estradiol and Progesterone Plasma Levels to Confirm Phase of Menstrual Cycle

Range of Normals	Day 1 of Menses E_2 very low (≤200 pmol/L) P very low (≤4 nmol/L)		Midfollicular Phase E_2 low but about double day 1 level (150 - 350 pmol/L) P very low (≤4 nmol/L)		Midluteal Phase E_2 elevated generally above midfollicular level 300 - 700 pmol/L) P very elevated (30 - 80 nmol/L)	
Subjects 2 cycles	E_2	P	E_2	P	E_2	P
#1	170		390		350	
	140		250		620	
		4		1		36
		<1		<1		56
#2	55		140		470	
	37		250		490	
		2		1		48
		<1		2		52
#3	140		220		370	
	90		360		300	
		1		4		31
		2		4		33
#4	120		230		290	
	150		ND		ND	
		4		1		32
		<1		ND		ND
#5	70		150		300	
	ND		150		310	
		1		1		27
		ND		<1		33
#6	55		340		430	
	50		170		520	
		1		<1		38
		<1		<1		42
#7	100		360		300	
	110		220		390	
		2		<1		68
		3		<1		63
#8	90		210		550	
	160		210		510	
		2		1		62
		1		<1		31

Table 2b

Estradiol and Progesterone Plasma Levels to Confirm Phase of Menstrual Cycle

Range of Normals	Day 1 of Menses E_2 very low (≤200 pmol/L) P very low (≤4 nmol/L)		Midfollicular Phase E_2 low but about double day 1 level (150 - 350 pmol/L) P very low (≤4 nmol/L)		Midluteal Phase E_2 elevated generally above midfollicular level 300 - 700 pmol/L) P very elevated (30 - 80 nmol/L)	
Subjects 2 cycles	E_2	P	E_2	P	E_2	P
#9	50		185		300	
	20		150		350	
		1		1		32
		3		1		34
#10	110		270		300	
	ND		ND		1100	
		<1		<1		40
		ND		ND		53
#11	120		340		500	
	65		240		340	
		1		<1		104
		1		1		50
#12	100		ND		310	
	35		150		350	
		1		<1		48
		<1		<1		62
#13	55		180		310	
	170		230		320	
		4		1		61
		2		<1		52
#14	40		180		270	
	120		250		450	
		1		<1		31
		<1		<1		25
#15*	25		110		80	
	60		140		45	
		<1		1		1
		<1		<1		1

*Subject using oral contraceptive

Determination of Maximum Work Capacity and Ratings of Perceived Exertion

Each subject's perception of stress experienced (RPE) during the 12 minute run are recorded in Table 3 for runs made on first day, midfollicular, and midluteal phases of two menstrual cycles. The determined maximum work capacity and treadmill settings for the 12 minute run assigned each subject are recorded in Table 4.

Maximal Run Time (MRT)

The individualized work loads used during the 12 minute run and the run to exhaustion are shown in Table 4. The maximum running times (MRT) at 100% of work capacity for each subject are shown in Table 5. The changes in MRT of each subject as they progressed through their cycles are illustrated in Figures 3a to 3c. The lines are drawn through the averages of running times at corresponding phases of each of the two menstrual cycles. Variations of run times found in each of two cycles are illustrated by vertical bars. To check for a possible relationship between the estradiol level for each subject and her MRT, comparisons of MRT were made at day 1 when the estradiol level was lowest, and at the midfollicular and midluteal phases when the estradiol levels were highest.

Table 3

Perceived Exertion at Minutes Into Submaximal Run at Day 1, Midfollicular and Midluteal Phases

Subject	Phase of Cycle	Perceived Exertion at Minutes Into Submaximal Run											
		2		4		6		8		10		12	
# 1	Day 1	9	7	11	8	13	8	14	11	15	12	16	13
	Midfollicular	9	7	11	8	13	9	13	10	13	11	14	12
	Midluteal	7	7	9	9	10	10	11	11	12	12	13	13
# 2	Day 1	7	7	7.5	7	8	7	8	11	9	11	10	11.5
	Midfollicular	9	7	9	7	10	8	12	8	13	8.5	13	9.5
	Midluteal	7	7	7.5	7	8	7	9	7.5	10	8	10	9
# 3	Day 1	7	9	10	10	11	12	12	13	12	14	13	14
	Midfollicular	8	9	10	10	11	12	12	13	13	14	13	15
	Midluteal	9	8	12	11	13	12	14	14	15	14	15	14
# 4	Day 1	6	6	7	8	8	9	11	12	12	13	12	14
	Midfollicular	6		7		7		10		11		13	
	Midluteal	6		8		10		13		13		14	
# 5	Day 1	10		11		13		14		16		17	
	Midfollicular	7	7	11	9	13	10	15	13	17	15	17	16
	Midluteal	7	7	10	9	12	10	14	13	15	15	16	16
# 6	Day 1	7	7	8	8	9	9	12	11	12	12	12	12.5
	Midfollicular	8	7	9	9	10	10	11	12	12	12.5	12	13
	Midluteal	11	7	12	8	12	9	13	11.5	14	12	14	13
# 7	Day 1	7	7	9	9.5	11	11	13.5	14	16	15	18	16.5
	Midfollicular	7	7	11	9	12.5	11	14.5	13.5	16	14.5	17	16
	Midluteal	7	7	9	9	10.5	10	14	14.5	15	16	16.5	17
# 8	Day 1	10	11	13	13	13	13	15	15	15	16	15	16
	Midfollicular	10	10	12	10	12	12	14	13	15	14	16	14
	Midluteal	12	11	14	13	15	13	15	15	16.5	15	16.5	15
# 9	Day 1	9	8	12	10	14	13	16	14	16	15	16	15
	Midfollicular	8	8	11	10	13	13	15	14	16	16	17	16
	Midluteal	7	7	10	9	13	13	14	15	15	15	16	15
#10	Day 1	6		7		8		9		10		11	
	Midfollicular	6		8		8		10		10		12	
	Midluteal	7	6	8	7	8.5	8	11	10	13	11	13	12
#11	Day 1	7	6	7	7	9	7	10	9	11	11	12	12
	Midfollicular	6	6	7	7	8	8	10	9	11	11	12	12
	Midluteal	8	6	9	7	10	7	11	8	12	9	13	10
#12	Day 1	7		9		11		13		13		15	
	Midfollicular	7	7	9	9	10	10	11	12	13	13	13	14
	Midluteal	8	8	11	10	13	10	14	12	15	13	16	14
#13	Day 1	8	8	9	10.5	11	11	13	13	15	14.5	17	16.5
	Midfollicular	9	7	10	8	11.5	9	12	11.5	13	13	14	14
	Midluteal	9	7	11	8	12	10.5	14	12	15	13.5	16	14.5
#14	Day 1	7	7	9	10	10	11	13	14	14.5	16	15.5	16
	Midfollicular	7	7	10	10	11	11	13	13.5	14.5	15	15	16
	Midluteal	7	7	7.5	8	8	9	10	11	11	12.5	11	13
#15*	Day 1	9	9	10	11	11	13	12	14	13	15	15	16
	Midfollicular	7	9	9	11	12	12	13	14	15	15	16	15
	Midluteal	10	9	13	11	15	13	18	15	18	16	19	17

*On birth control pills

Table 4

Personal Data, Max MET, and Submaximal Work

Load Assignments for Measures of RPE

Subject Athletic involvement	Age	Max MET	Predicted MVO_2 ml/kg/min	Work Load Assignment								Maximum Heart Rate
				100% MET		60% MET		70% MET		80% MET		
				MPH	Incline	MPH	Incline	MPH	Incline	MPH	Incline	
#1. X-country skiing, Jogging 3x/week	33	11.2	39.2	5.0	7.5°	4.0	0	4.6	0	5.2	0	190
#2. Jogging, Club Volleyball 4x/week	18	11.8	41.3	5.0	10.0°	4.4	0	5.0	0	5.8	0	194
#3. Jogging 4x/week	33	12.9	45.2	5.0	12.5°	48	0	5.4	0	6.2	0	190
#4. Jogging, X-country skiing, 4x/week	28	12.9	45.2	5.0	12.5°	4.8	0	5.4	0	6.2	0	192
#5. Rower, Downhill skier 4x/week	19	13.2	46.2	5.5	10.0°	5.0	0	5.6	0	6.5	0	193
#6. Jogger 4x/week	28	13.2	46.2	5.5	10.0°	5.0	0	5.6	0	6.5	0	190
#7. Distance Runner 5x/week	32	13.7	47.9									185
#8. Rower, X-country Skiing 5x/week	22	15.1	52.5	5.5	12.5°	5.2	0	6.0	0	6.5	2.5°	196
#9. Jogger, Ringette 5x/week	20	12.9	45.2	5.0	12.5°	4.8	0	5.4	0	6.2	0	192

Table 4 (cont'd)

Personal Data, Max MET, and Submaximal Work

Load Assignments for Measures of RPE

Subject Athletic involvement	Age	Max MET	Predicted MVO_2 ml/kg/min	Work Load Assignment								Maximum Heart Rate
				100% MET		60% MET		70% MET		80% MET		
				MPH	Incline	MPH	Incline	MPH	Incline	MPH	Incline	
#10. Aerobics Instructor 4x/week	21	12.9	45.2	5.0	12.5°	4.8	0	5.4	0	6.2	0	182
#11. Club Volleyball 3x/week	20	12.9	45.2	5.0	12.5°	4.8	0	5.4	0	6.2	0	192
#12. Competitive Downhill skier 4x/week	17	12.9	45.2	5.0	12.5°	4.8	0	5.4	0	6.2	0	202
#13. Jogger 4x/week	29	13.2	46.2	5.5	10.0°	5.0	0	5.6	0	6.5	0	207
#14. Distance Runner 4x/week	22	15.3	53.6	7.0	7.5	5.5	0	6.4	0	6.6	2.5	204
#15.* Recreational Jogger	22	12.0	42.0	5.0	7.5°	4.2	0	4.8	0	5.5	0	194

*on oral contraceptives

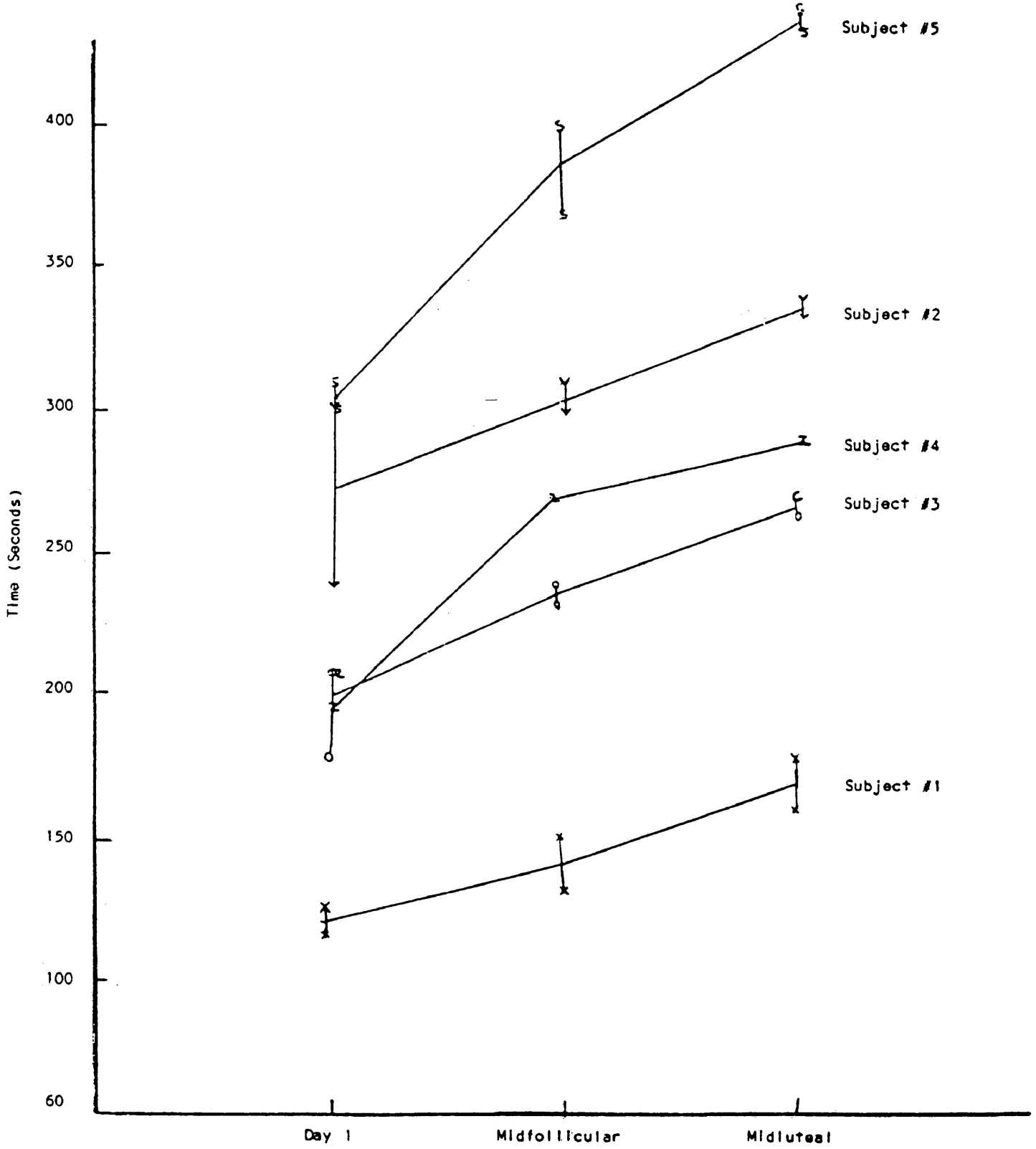

Figure 3a. Run times to voluntary exhaustion at 100% work capacity at Day 1, Midfollicular and Midluteal phases of the menstrual cycle.
Lines drawn through means of values found on two consecutive cycles.

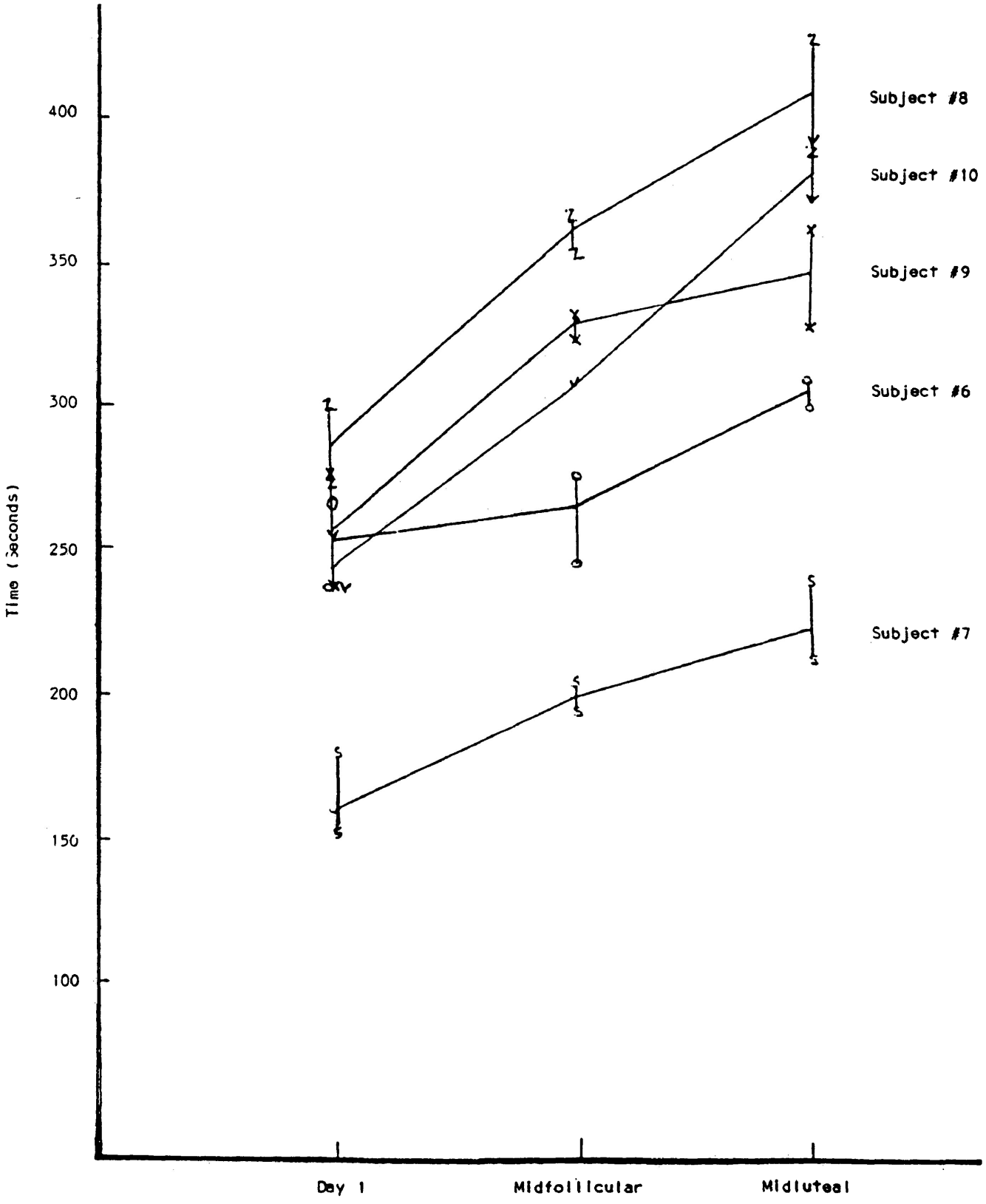

Figure 3b. Run times to voluntary exhaustion at 100% work capacity at Day 1, Midfollicular and Midluteal phases of the menstrual cycle.
Lines drawn through means of values found on two consecutive cycles.

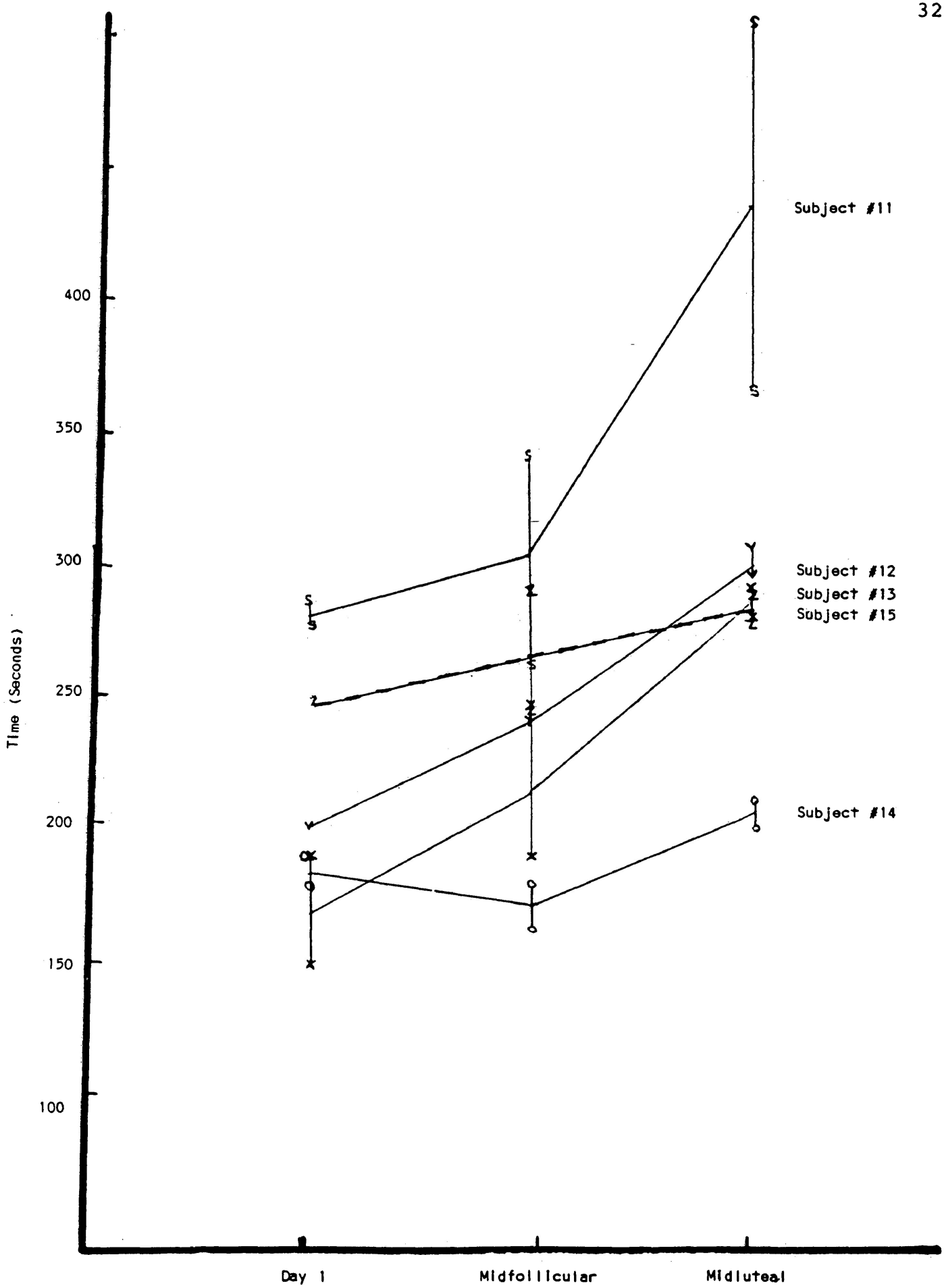

Figure 3c. Run times to voluntary exhaustion at 100% work capacity at Day 1, Midfollicular and Midluteal phases of the menstrual cycle. Subject 15 was using oral contraceptives.

Lines drawn through means of values found on two consecutive cycles.

Table 5

Maximal Run Time and Phase of Cycle

Subject	Maximum Run Time (seconds)						% Mean Increase in Maximum Run Time		
	Day 1		Midfollicular		Midluteal		Midfollicular vs Day 1	Midluteal vs Day 1	Midluteal Midfollicular
1	242	313	311	305	341	338	10.8	22.3	10.4
2	132	122	157	137	185	168	15.7	38.5	19.7
3	201	214	246	242	268	275	18.3	30.9	10.6
4	185	216	277	ND	293	ND	37.8	45.8	5.8
5	307	ND	407	372	445	437	26.7	43.6	13.4
6	242	268	256	277	310	306	2.3	20.8	18.0
7	156	180	203	205	215	240	21.4	35.7	11.8
8	271	303	365	361	393	431	26.5	43.6	13.5
9	273	241	330	336	331	366	29.6	35.4	4.5
10	248	ND	307	ND	398	379	23.8	56.8	26.7
11	276	286	264	345	366	547	8.2	60.1	48.0
12	199	ND	240	240	294	306	20.6	50.7	25.0
13	188	147	184	243	271	285	28.1	66.5	29.9
14	180	88	180	164	207	203	-6.5	11.4	19.2
Mean: 1-14	226.1		260.9		317.1		18.8	40.2	17.9
15*	247	241	286	242	283	286	6.8	15.4	8.0

*on oral contraceptives

Blood LA levels were compared at each phase of the cycle.

A summary of the ANOVA one-way analysis of variance with each phase of the cycle for each subject's mean MRT, mean RPE, mean LA, mean E-L and mean P is shown in Table 7.

Table 6

Lactic Acid Levels mmol/L at rest, After Submaximal Run, and After Run to Voluntary Exhaustion at Day 1, Midfollicular and Midluteal Phases of the Normal Cycle of 15 Subjects.

		Day 1		Midfollicular		Midluteal	
Subject #1	- Rest	.8	1.3	1	1.4	0.9	0.8
	Sub Max	2.1	1.6	1.6	1.2	1.3	1.6
	Max	7.5	6.8	7.3	7.1	8.6	7.2
Subject #2	- Rest	1.0	1.2	1.4	1.1	.7	1.0
	Sub Max	3.1	2.9	2.3	2.5	3.2	2.4
	Max	9.6	9.4	8.2	7.1	7.6**	8.6
Subject #3	- Rest	1.0	1.0	1.8	1.4	1.7	1.6
	Sub Max	1.8	2.1	2.8	1.8	1.2	2.1
	Max	7.2	6.2	9.1	9.2	8.2	6.9**
Subject #4	- Rest	.8	1.0	1.1	ND***	1.3	ND
	Sub Max	1.8	2.3	1.8	ND	1.8	ND
	Max	12.7	11.4	10.9	ND	10.3	ND
Subject #5	- Rest	2.3	ND	0.9	0.9	0.8	0.8
	Sub Max	2.9	ND	2.0	1.8	1.8	1.9
	Max	7.0	ND	7.8	7.6	6.9	6.6
Subject #6	- Rest	1.1	0.4	0.9	0.7	0.7	0.4
	Sub Max	1.8	1.8	1.7	1.7	2.0	3.6
	Max	9.8	9.9	8.5	9.1	6.0**	10.1
Subject #7	- Rest	0.7	0.9	1.8	2.1	1.1	1.1
	Sub Max	6.2	6.2	7.6	5.3	7.5	5.4
	Max	6.3	10.2	11.3	9.2	8.1	8.0
Subject #8	- Rest	1.2	1.3	1.3	0.8	1.1	1.0
	Sub Max	1.7	1.8	1.4	0.9	1.3	1.7
	Max	11.7	8.4	8.3	11.1	7.0	7.1
Subject #9	- Rest	0.9	0.6	1.0	0.9	0.9	1.0
	Sub Max	3.4	3.4	3.7	3.5	4.6	4.1
	Max	11.5	8.0	10.1	9.1	14.3	13.4
Subject #10	- Rest	ND	1.0	0.7	ND	0.8	1.1
	Sub Max	ND	1.2	1.0	ND	1.3	1.3
	Max	ND	8.0	7.9	ND	8.4	8.1
Subject #11	- Rest	0.7	0.7	1.0	0.7	0.8	0.7
	Sub Max	1.1	1.0	1.7	1.0	1.6	1.0
	Max	8.8	10.2	8.5	8.9	6.7**	10.9
Subject #12	- Rest	1.0	ND	1.6	0.6	1.1	1.0
	Sub Max	1.3	ND	1.7	1.5	1.6	2.6
	Max	9.3	ND	10.6	9.7	9.8	10.1
Subject #13	- Rest	0.8	0.6	0.7	0.7	0.9	0.8
	Sub Max	2.4	4.7	2.8	3.9	3.6	3.4
	Max	7.6	7.4	10.0	8.7	8.2	8.1
Subject #14	- Rest	0.7	0.9	0.7	0.8	1.6	0.9
	Sub Max	2.6	3.2	2.0	4.2	2.7	3.2
	Max	9.8	8.6	9.9	8.1	10.3	ND
Subject #15*	- Rest	0.8	0.7	0.9	0.8	0.8	0.7
	Sub Max	2.0	2.4	1.5	2.2	2.3	2.8
	Max	7.8	7.9	8.4	8.7	10.6	10.0

*on oral contraceptives
**late blood sample
***No Data (ND)

Table 7

One Way Analysis of Variance of RPE, MRT, Lactic Acids, Estradiol and Progesterone with Phase of Menstrual Cycle

Results-Means, F & P

Variable	Day 1	Midfollicular	Midluteal	F	P
RPE 7 (minute)	11.82	11.51	11.82	.247	.7823
8	12.37	11.97	12.42	.1355	.8736
9	12.88	12.68	13.06	.5532	.946
10	13.43	13.20	13.24	.5361	.91
11	13.89	13.66	13.59	.3124	.861
12	14.36	14.04	14.07	.123	.88
Max Run Time (second)	226.1	268.9	317.1	7.048	.0024
LA mmol/L					
1 (resting)	1.02	1.13	1.04	.385	.683
2 (post sub max)	2.47	2.39	2.55	.164	.9525
3 (post max)	8.94	8.96	9.24	.1825	.8338
E_2 pmol/L					
1 (resting)	98.14	223.71	392.9	31.22	.0000
2 (post sub max)	94.79	260.71	472.3	33.85	.0000
3 (post max)	130.36	289.64	548.9	42.97	.0000
P nmol/L					
1 (resting)	2.14	1.07	42.5	200.57	.0000
2 (post sub max)	2.0	1.0	52.3	88.31	.0000
3 (post max)	1.93	1.29	180.4	90.22	.0000

CHAPTER V

DISCUSSION

The purpose of this study was to determine whether there was any relationship between athletic performance and the biochemically confirmed phases of the menstrual cycle in female athletes.

A number of studies have found that a large percentage of participants perform "less well" while menstruating (Dalton, 1960; Erdelyi, 1976; Higgs and Robertson, 1981; Moore and Barker, 1923; Wearing et al, 1972;). There is less agreement in the literature whether any one phase of the cycle favours maximal performance. It has been suggested that errors in assigning phases may have masked any relationship (Doolittle, 1972). Jurkowski et al (1978) were the first to use hormonal levels to confirm the cycle phases and relate them to work capacity. They confirmed the midfollicular (6 - 9 days after onset of menstruation) and the midluteal phase (6 - 9 days after ovulation) by measurement of plasma progesterone. Ovulation was indicated by a sustained increase of at least 0.3°C in basal body temperature. In the midfollicular phase the plasma levels of P are very low and generally equal to or less than 4 nmol/L. At the same time, E-2 levels are only moderately elevated and generally range between 150 and 350 pmol/L. In the midluteal phase, plasma levels of P are much higher and generally exceed 30 nmol/L while levels of E-2 are about

double those in the midfollicular phase and generally exceed 300 pmol/L (Henry, 1979).

In this study measurements of P and E-2 were carried out at day 1, day 7±, and day 21± of the cycle before work tasks were attempted. When the resting E_2 and P blood levels were not within expected limits for the midfollicular (day 7) and midluteal (day 21) phases, (see Tables 2a and 2b), the performance measures were rejected and tests were repeated at a later menstrual cycle. Some 12 data sets were rejected because measures of E-2 or P were outside the limits. There were 7 subjects who had an E-2 level considerably below the 150 pmd/L required for the midfollicular phase, and 5 subjects who had P levels below 30 nmol/L required for the midluteal phase. Retesting of these individuals on subsequent cycles yielded E-2 and P levels within the general expected range. One participant #15, known to be taking oral contraceptives, had relatively stable levels of E-2 and P throughout her cycle. Her phase of cycle therefore could not be confirmed by hormone assay. For her tests, the data were collected at day 7 and day 21, as if the phases were correctly assigned.

The perceived exertion of each of the subjects carrying out a submaximal task was found to be unaffected by the phase of menstrual cycle. The mean RPE at minute 12 on day 1 was 14.36, for the midfollicular phase 14.04, and for the midluteal phase it was 14.07. This non-effect of phase on

RPE for submaximal work was confirmed by a one-way analysis of variance set out in Table 6. This is in agreement with Stephenson et al (1982), who found no statistically significant changes in RPE at any exercise intensity related to the cycle phase of healthy women.

These observations are not in conflict with those of Gamberale et al (1975) who found that subjects who suffered severe menstrual distress, reported greater RPE while carrying out submaximal work loads during menses than during pre- or post-menstrual periods. Unlike those of Gamberale, subjects in this study experienced 'normal' premenstrual tension and discomfort and were able to carry out assigned tasks without significant discomfort on day 1 of menses.

Lactic acid accumlations may help to explain the insignificant changes in RPE when performing a standard task at different phases of the menstrual cycle. Lactic acid, present in the blood in the form of lactate, is the end product of anaerobic metabolism, and its level is inversely related to oxygen availability. Morgan (1973) believes lactic acid accumulation plays a major role in the perception of muscle fatigue. After having reached exhaustion, a delay of a few minutes is necessary before levels in circulating blood reflect the rate of diffusion from working muscles (Burke, Fleck and Dickson, 1981; De Bruyn-Prevost and Sturbois, 1980; Diamont, Kalson and Saltin, 1968). Diamont et al (1968) and Burk et al (1981) recommend measures of lactic acid at 2 and 5 minutes after completion of a work task.

Table 6 indicates that no significant differences were observed in lactic acid levels produced by either submaximal or maximal work in any of the three phases of the cycle. Mean levels of lactic acid 2 minutes after the submaximal run were 2.47, 2.39 and 2.54 mmol/L for day 1, midfollicular and midluteal phases respectively, and mean lactic acid levels 5 minutes after the run-to-exhaustion were 8.94, 8.96 and 9.24 mmol/L for day 1, midfollicular and midluteal phases respectively.

The insignificant difference in lactic acid production in any phase of the menstrual cycle following the run to exhaustion, is contrary to that found by Jurkowski (1982). She observed that plasma lactic acid levels produced by heavy exercise in the follicular phase were higher than levels produced by the same exercise in the luteal phase. These differences persisted for at least 5 minutes into the recovery phase. The heavy exercise reported by Jurkowski consisted of 20 minutes of exercise at 60-70% of the individual's work capacity. Blood samples were collected from an indwelling catheter during the exercise, as well as at 5 and 10 minutes into recovery. Only after 10 to 15 minutes into the heavy exercise did levels of plasma lactate in the follicular phase significantly increase above the level in the luteal phase.

The 6 minute period of work at 80% of capacity in this study may not have been long enough for influence of phase to affect the lactic acid level.

Findings of other investigations support the conclusion that RPE for submaximal work load is not influenced by phase of cycle. Higgs and Robertson (1981) found no statistical difference in RPE for day 1 versus midcycle performance of work at 90% VO_2 max. Only when performing at 100% VO_2 Max did their subjects register a significant increase in RPE at day 1 over the RPE at midcycle. Stephenson et al (1982) found no significant changes in RPE at any exercise intensity that could be related to cycle phase.

An interesting finding was the increase in maximum run times (MRT) to exhaustion in the midluteal phase in comparison to MRT in either the midfollicular phase or day 1 of menses. The average increase in MRT's in the midluteal phase over the MRT's of day 1 was 15.4%. The average increase in MRT's in the midfollicular phase over the MRT's of day 1 was 6.8%. The only variation from this pattern was by subject #14, who recorded her poorest average run time during the midfollicular phase, and her best average MRT during the midluteal phase. The same pattern held true for subject #15 on oral contraceptives whose MRT in the midluteal phase was 8.0% higher than in the midfollicular phase.

Less surprising was the average increase of 18.8% in MRT made in the midfollicular phase compared to MRT's registered on day 1 of menses. For subjects 1 to 14, the mean MRT was 268.9 seconds in the midfollicular phase versus 226.1 seconds on day 1. A number of authors have observed impaired performance immediately prior to, and/or on first day of menstruation. Erdelyi (1962) reported extremely poor

performance in tennis and rowing during menstrual periods, but unimpaired performance in track and field. Erdelyi went so far as to recommend that it was not adviseable to take part in any training or contests during mentruation. Doolittle and Engebretsen (1972) reported no cycle related change in three endurance performance tests which included a 12 minute run, a 600 yard run, and a 1.5 mile run.

The findings of this study suggest an impaired performance of 13 of 14 athletes running to voluntary exhaustion on the first day of menses. Only one subject, number 14, had a 6.5% longer MRT on day 1 of menses than that at the midfollicular phase. It should be observed that subject #15 who was taking oral contraceptives did not suffer a noticeably poorer performance at day 1 as compared to her performance during other phases of her cycle.

Jurkowski (1982) recorded a two-fold increase in time to exhaustion at 90% Wmax in the luteal phase over the follicular phase. This study found a significant but smaller difference in time-to-exhaustion at 100% work capacity in the two phases. The physical condition of Jurkowski's 'physical education students' was possibly less than that of the competitive athletes in this study.

The finding of a consistent increase in the MRT of athletes in the midfollicular and the midluteal phase over day 1, may have important implications for preparations for strenuous competition. One usually has no influence over the date of a competition, nor is it practical to attempt to postpone the onset of menstruation. The data of subject #15

suggests that the use of oral contraceptives might level out the performance over the three phases of the menstrual cycle. It remains to be determined whether their use reduces the performance in the midluteal phase to the performance level of day 1, or whether it elevates the performances on day 1 to the level in the midluteal phase. Such might well be the subject of a future study on female athletes maintaining a training program before and after their acceptance of oral contraceptives on a regular schedule.

CHAPTER VI

SUMMARY AND RECOMMENDATIONS

Summary

The purpose of this study was to identify and confirm biochemically the phases of the menstrual cycle in well-conditioned athletes and then to examine the effects of the various phases on work capacity, perceived exertion and lactic acid accumulation following individualized work tasks. Radioimmunoassays of plasma estradiol and progesterone were used to confirm the midluteal and midfollicular phase of cycle. The phases in which tests were carried out were day 1 of flow, the midfollicular phase and the midluteal phase. The work load assigned to each subject was based on her individual maximum work capacity as determined by the Balke Treadmill Test. Perceived exertion was recorded using the Borg Scale while subjects completed a 12 minute run, 3 minutes at each of 60% and 70% of their maximum work capacity and the last 6 minutes at 80% of maximum work capacity. After a fifteen minute rest the subjects were asked to run as long as they could at a work rate corresponding to 100% of their maximum work capacity.

Blood samples were drawn for lactic acid assays prior to the start of the initial run, 2 minutes after the submaximal run and again 5 minutes after the run to exhaustion.

Fourteen healthy well conditioned athletes with 'normal cycles' and under no medication comprised the test group. A fifteenth athlete was also tested although she was regularly

taking oral contraceptives. Subjects were tested at each phase of 2 cycles in order to compare cycle to cycle scores. Data was obtained for perceived exertion, maximum run time and blood lactic acid levels.

The phase of the cycles had no affect on perceived exertion or on the accumulation of lactic acid in any subject performing at 80% and 100% of her work capacity.

The most apparent difference was the increase in the maximum run times. All subjects had improved run times in the midfollicular and midluteal phases over the run time on day 1 of flow. Also the run times in the midluteal phases were significantly greater than the run times in the mid-follicular phases. These differences were statistically significant at the $P<0.05$ level.

No relationship could be found between the change in estradiol levels on day 1 and during the midluteal phase that could account for the subjects' ability to perform better in the midluteal phase.

Recommendations

This author recommends that:

A prospective trial be made to determine whether the adoption of an oral contraceptive regime would allow a subject to increase her work capacity on day 1 of menses to the level attained in the midluteal phase before the oral contraceptive was adopted, or whether the regular use of an oral contraceptive would limit the midluteal performance to that of Day 1 of flow. The information obtained from such a study might have important implications for preparation of female athletes for strenuous competitive events.

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APPENDIX A

Questionnaire On Menstrual Cycle

You have indicated interest in participating in a proposed research study concerning certain aspects of performnce and the menstrual cycle. Your assistance would be of great importance and would be deeply appreciated. The time required for participation will be approximately eight hours over a two to three month period. Under these conditions would you be willing to volunteer as a subject for this study?

CHECK: _____ Yes _____ No

The following information is essential in determining the final selection of subjects. All information given will be considered strictly confidential.

NAME: _____ _____ BIRTHDATE: month/year: __,__

PHONE NUMBER: _____

1. Is your menstrual period regular? _____ Yes _____ No
   a) Number of days between the onset of two successive menstrual periods. _____
   b) Average length of your menstrual period. _____
   c) Anticipated date of your next menstrual period. _____

2. Do you experience discomfort during your menstrual period?
   _____ Yes _____ No
   a) Check any of the following discomforts which you experience:

   _____ Abdominal cramps Others: _____
   _____ Backache _____
   _____ General discomfort _____
   _____ Unusual tension _____
   _____ Headache
   _____ Leg cramps
   _____ Tiredness

   b) Discomfort during first day. _____ Yes _____ No
   c) Discomfort during first and second day.
      _____ Yes
      _____ No

3. Do you usually decrease the amount of physical activity during the first day of your menstrual period?
   _____ Yes
   _____ No

Thank you!

HUMAN PERFORMANCE ASSESSMENT CENTRE

Department of Physical Education

University of Regina

CLIENT HISTORY QUESTIONNAIRE A
To Be Completed by Physician

FOR PARTICIPATION IN GENERAL FITNESS ASSESSMENT

PATIENT'S NAME _____ DATE _____
Last First Initial

ADDRESS _____ PHONE _____ _____

BIRTH DATE ____ / ____ / ____ AGE _____
Day Month Year

I consider the above individual as: _____ "Normal"

_____ Cardiac Patient

_____ Prone to Coronary Heart Disease

_____ Other (Explain) _____

_____

_____

A) DIAGNOSTIC DATA ETIOLOGIC

1. No heart disease
2. Rheumatic heart disease
3. Congenital heart disease
4. Hypertension
5. Ischemic heart disease
6. Heart Murmurs or Gallops

_____

7. Other _____

_____

_____

B) PRESENT PHYSICAL ACTIVITY

1. Very Active
2. Moderately Active
3. Active
4. Inactive
5. Restricted Activities
6. Sedentary

C) ECG

1. Within Normal Limits
2. Drug Effect Only (eg. digitalis)
3. Abnormal
   a) Non Specific
   b) Previous Infarct
4. Other _____

D) RHYTHMN

1. Sinus
2. Arrhythmia (Type and Treatment)

   _____

   _____
3. Pacemaker Type _____

E) SPECIFIC CARDIAC DIAGNOSIS _____ NONE _____

II. ANY ADDITIONAL ABNORMALITIES YOU ARE AWARE OF AND DATE DIAGNOSED _____

III. PRESENT MEDICATIONS OR TREATMENTS _____ _____

IV. PREVIOUS SIGNIFICANT MEDICAL HISTORY (PLEASE COMMENT)

ACCIDENTS _____ _____

ALLERGIES _____ _____

DIABETES _____ _____

EPILEPSY _____ _____

INFECTIONS _____ _____

LUNG DISEASE _____ _____

_____

MENTAL ILLNESS _____ _____

_____

NEURAL IMPAIRMENT _____ _____

_____

TUMORS _____ _____

_____

V. Please fill in the information below if it is available:

1. Blood count: Hbg. _____

2. ECG, 12 lead (enclose copy) _____

3. Blood pressure, Syst._____ Diast. _____ (Medicated _____ Unmedicated _____)

4. Glucose _____ mg.%

5. Cholestrerql _____ mg.%

   Fasting Triglyceride _____ mg.%

6. Graded Exercise Test Results (if available, enclose)

Impression of above information _____

_____

VI) DATE OF LAST COMPLETE PHYSICAL EXAM AND DIAGNOSIS _____

_____

APPENDIX B

Borg's 15 Point Scale for Ratings of Perceived Exertion

6.
7. Very, very light
8.
9. Very light
10.
11. Fairly light
12.
13. Somewhat hard
14.
15. Hard
16.
17. Very hard
18.
19. Very, very hard
20.

APPENDIX C

immuno-ESTRADIOL iodine-125 kit

PANTEX 047

I. Intended use

Pantex immuno- $^{1}_{-}$ $^{2}_{-}$ ^{5}I kits are designed for quantitive serum analysis by properly qualified laboratory personnel. They are used only "For in vitro diagnostic use."

II. Principles of the method

This radioimmunoassay method depends upon competition between the radio-labeled ligand in the tracer reagent and unlabeled ligand in the standard, control or sample for a constant and limiting concentration of antibody binding sites provided in the 1st antiserum. The unlabeled ligand in the incubation mixture reduces the amount of labeled ligand bound to the antibody. There is a quantitative relationship between the concentration of unlabeled ligand in the sample or standard and the proportion of tracer bound to the antibody. The ligand-antibody complex (the bound) is precipitated by the 2nd antiserum. Counts of the precipitated ligand are measured in a gamma counter after removal of the supernatant. Binding values of the standards are plotted vs. concentrations and the best line is drawn through the points. The binding values of the controls or samples are plotted on the line. These points indicate the ligand concentrations on the X-axis.

III. Procedure

Sample extraction

1. Add 600 microliters of samples to screw-cap tubes. Add 6.0 ml of ethyl acetate:hexane (3:2). Tighten Teflon-lined caps and shake vigorously for 60 seconds. Centrifuge the tubes, or simply allow layers to separate (approximately 5 minutes).
2. Transfer 5.0 ml of each upper layer into glass scintillation vials or tubes. Be careful not to contaminate them with any of the lower layer. Evaporate extracts to dryness with air or nitrogen. Use of a 40-50°C bath will increase the rate of evaporation.
3. To each vial add 1.25 ml of diluent. Cover. Let stand for 30 minutes at 37°C and mix before analysis. [Each 0.5 ml of reconstituted extract (recon. extr.) contains the equivalent of 200 microliters of the original serum sample.]
4. Use of 1.2 or 1.8 ml of serum will give greater sensitivity for samples with low E2 concentrations (e.g. prepubertal or postmenopausal). An additional factor is required in the calculation: see VIII.5.

Standard dilution

Since standards in buffer are not stable for extended periods, dilutions must be made just prior to each run. Pipet carefully in all the following steps. Pipet 100 microliters of stock standard into a 10 ml volumetric flask. Add diluent up to 10 ml. Mix. The E2 concentration is 512 pg/ml: equivalent to 1280 pg of E2/ml of serum in samples.

To each of 7 tubes labeled 640, 320, 160, 80, 40, 20, and 10 add 2.0 ml of diluent. Make serial 2-fold dilutions by pipetting 2.0 ml of the 1280 pg/ml standard to the 640 tube. After mixing, pipet 2.0 ml from the 640 tube to the 320 tube, etc. Concentrations of all these diluted standards are expressed as their serum equivalents. The actual weight of E2 pipetted into tubes is

indicated in Table 1.

RIA procedure

Allow reagents to come to room temperature and mix them gently just before use. See the flow sheet in Table 1. Label tubes for: total count, non-specific binding (NSB), standards, control sera and unknowns. The number of standard and sample replicates per run is determined by each laboratory. Since the total count tubes contain only the tracer, these may be capped or stoppered and recounted for subsequent assays in which the same bottle of tracer is used.

The reagents are color coded. After addition of reagent at each step all tubes will have the same color, if the proper reagents have been added to each tube. The proper mixed color is indicated below and also on the flow sheet. It is obvious if a reagent has not been added to one of the tubes. The total count tubes are the only exceptions: they remain yellow since only the yellow tracer is added to them.

1. Pipet 500 microliters of diluted standards or recon. extr. of samples into the tubes. Add 500 microliters of diluent to NSB and zero std. tubes.

tubes-colorless

2. Pipet 100 microliters of diluted tracer into each tube.

tubes-yellow

3. Pipet 100 microliters of 1st antiserum into each tube, except

to total count and NSB tubes.

tubes-green

4. Add 100 microliters of NSB buffer to each NSB tube.

tubes-green

4a. Mix tubes gently. Incubate tubes at 37 C for a minimum of 30 minutes.

5. Add 500 microliters of 2nd antiserum to each tube, except to total count tubes.

tubes-brown

6. Mix tubes gently and leave them at room temperature for 10 minutes.

7. Centrifuge tubes at 2500 RCF x G for 10 minutes. Aspirate or decant and discard each supernatant without disturbing the precitipate. Absorb any liquid on the lip of the tube with tissue. Any liquid left in the tube included unbound radioactivity. If an appreciable volume of liquid remains, an apparent high B/B0 value will be obtained. This could cause low values at the lowest ligand levels.

8. Count all tubes: total; and precipitates of NSB, standards, controls, and unknowns. Determine the background count.

IV. Calculation

Subtract the mean NSB count from each corrected count (except

total) to obtain net counts. (Corrected count is the gross count minus background.) Counts used in the calculation below are net counts. As an example of data calculation, see Table 2, the data table from an experimental run, and Fig. 1, on which the results of standards are plotted. Calculation using the logit-log plot (17) is as follows:

1. Calculate the relative percent bound (%B/B0) of each standard, i.e. of each standard relative to the zero standard.

$$\%B/B0 = \frac{\text{counts of standard}}{\text{counts of zero standard}} \times 100$$

2. Plot on logit-log graph paper the points relating these calculated %B/B0 values and standard concentrations. Draw the best line through the points.

3. Calculate %B/B0 of the controls and samples.

$$\%B/B0 = \frac{\text{counts of unknown}}{\text{counts of zero standard}} \times 100$$

4. Plot the %B/B0 values on the line. Project these points to the X-axis to obtain the ligand concentrations.

5. When volumes of serum either greater or less than 600 microliters have been extracted, the E2 concentration obtained from VIII.4 must be multiplied by a factor:

$$\text{factor} = \frac{600}{\text{microliters of serum extracted}}$$

or using the column extraction:

$$\text{factor} = \frac{500}{\text{microliters of serum added to the column}}$$

V. Limitations of the procedure

If an E2 value does not lie within the standard range, make an appropriate dilution of the recon. extr. with diluent and repeat the analysis. Multiply the concentration from VIII.4 or VIII.5 by the dilution factor to obtain the serum E2 level.

1. The cross reactivities of the antiserum were measured based on 50% displacement of labeled E2 by chemically related compounds.

2.

compound	relative activity (%)
estradiol-17beta	100
estradiol-17alpha	1.4
estriol	under 0.018

Compounds with values under 0.001% were: androstenedione, cholesterol, corticosterone, cortisol, DHEA, estrone, pregnenolone, orogesterone, testosterone.

3. Solvent loss before aliquotting in step 2 in the tube extraction should be minimized because evaporation of considerable solvent may result in a total volume less than 6 ml, the aliquotting of more than 5/6 of the solvent, and

therefore higher values. Tubes should not be allowed to stand without tops for extended periods after solvent is added or during aliquotting.

4. Solvent suitability It is desirable to check blanks and recoveries on new lots of the hexane and ethyl acetate used for extraction to assure that they don't contribute significant amounts of apparent E2 to samples of low concentration. If necessary, blank can be eliminated by redistilling the solvent in an all glass apparatus.

VI. Reference values

	serum E2 (pg/ml)
female	4.0-12.0
ovulating, normal	30-100
early follicular	100-400
late follicular	50-150
luteal phase	up to 35,000
pregnancy, normal	
hMG treatment:	350-750
therapeutic range	5.0-18.0
postmenopausal or castrate	under 50 (18,19)
on oral contraceptives	
male normal	
prepubertal	2.0-8.0
adult	10-60

APPENDIX D

No Extraction

Progesterone

Coat-A-Count Progesterone is a no extraction, solid phase ^{125}I radioimmunoassay designed for the quantitative measurement of progesterone in serum. It is intended strictly for in vitro use as an aid in clinical diagnosis.

Catalog Numbers: TKPG1 (100 tubes) TKPG2 (200 tubes)

The 100 tube kit contains not more than 4 microcuries (148 kilobecquerels) of radioactive ^{125}I Progesterone. The 200 tube kit contains not more than 8 microcuries (296 kilobecquerels).

Methodology

In the Coat-A-Count progesterone procedure, ^{125}I labeled progesterone competes with progesterone in the patient sample for sites on progesterone-specific antibody immobilized to the wall of a polypropylene tube. After incubation for a fixed time, isolation of the antibody-bound fraction is achieved simply by decanting the supernatant. The tube is then counted in a gamma counter, the counts being inversely related to the amount of progesterone present in the patient sample.

The coated tube, ^{125}I methodology offers significant gains in convenience compared to liquid-phase and tritiated systems, while maintaining the highest standards of accuracy and reliability. There is only one reagent to dispense, and no need for centrifugation. The kit features total counts of approximately 50,000 cpm(at iodination) and a maximum binding of approximately 45 to 50%. The calibrators, which are shipped

in liquid form, ready to use, have been prepared in processed human serum. The calibration curve is linear, in a logit-log representation, throughout the 0.1-40 ng/ml (0.3-127 nmol/l) range of the calibrators.

Reproducibility is excellent, as documented in the Performance Data section below. The assay is sensitive to as little as 0.05 ng/ml progesterone. The immobilized antiserum is highly specific for progesterone, as shown by the table of crossreactivities. Accuracy has been confirmed by recovery of progesterone from spiked human serum. The kit's freedom from 'matrix effects' is demonstrated by patient scaling experiments and by studies, presented in this protocol, on the effect of bilirubin, hemolysis and lipemia.

Radioimmunoassay Procedure

All components must be at normal room temperature prior to use.

1. Plain Tubes: Label four plain(uncoated) 12 x 75mm polypropylene tubes T (total counts) and NSB (nonspecific binding) in duplicate.

   Coated Tubes: Label fourteen Progesterone Antibody-Coated Tubes A (maximum binding) and B through G in duplicate. Label additional antibody-coated tubes, also in duplicate, for each control and patient sample.
2. Pipet 100 ul of the zero calibrator A into the NSB and A tubes, and 100 ul of each of the calibrators B through G into correspondingly labeled tubes. Pipet 100 ul of each control and patient sample into the tubes prepared.
3. Add 1.0 ml of Buffered [^{125}I] Progesterone to every tube. Vortex briefly and gently.
4. Incubate for 3 hours at room temperature.

5. Decant thoroughly.

6. Count for 1 minute in a gamma counter.

Calculation

To calculate progesterone concentrations from a logit-log representation of the calibration curve, first determine for each pair of tubes the average NSB-corrected counts per minute:

Net Counts = Average CPM minus Average NSB CPM

Then determine the binding of each pair of tubes as a percent of maximum binding (MB), with the NSB-corrected counts of the A tubes taken as 100%:

$$\text{Percent Bound} = \frac{\text{Net Counts}}{\text{Net MB Counts}} \times 100$$

Using the logit-log graph paper provided with the kit, plot Percent Bound on the vertical axis against Concentration on the horizontal axis for each of the calibrators B through G, and draw a straight line approximating the path of these six points. Progesterone concentrations for the unknowns may then be estimated from the line by interpolation.

Expected Values: Serum Progesterone

A 'normal range' study was performed at a large hospital laboratory using the Coat-A-Count Progesterone kit. The population was screened to exclude women with fertility problems. Note that the mid-luteal samples constitute a subset of the luteal phase group. The results are displayed below in ng/ml.

Reference Group	Median	Absolute Range	(n)
Males	0.1	0.0 - 0.4	(24)
Females:			
Follicular Phase	0.4	0.1- 1.5	(28)
Luteal Phase	8.5	2.5- 28.1	(22)
Mid-Luteal Phase	23.2	5.7- 28.1	(7)
Over 60 Years Old	0.1	0.0- 0.2	(20)
Oral Contraceptives	0.2	0.1- 0.3	(6)
Pregnant Females:			
First Trimester	24.5	9.0- 47.0	(32)
Second Trimester	60.0	16.8-146	(33)
Third Trimester	123	55.0-255	(32)

The data indicate that progesterone values transiently increase many fold during the middle of the luteal phase of the menstrual cycle. In pregnancy the general tendency is for values to increase. Ninety percent of the values for pregnancy fell in the ranges 13-35, 25-105, and 65-200 ng/ml for the first, second and third trimesters, respectively. There is considerable interpersonal variation in progesterone values particularly in groups associated with elevated levels. Here it should be stated again that many investigators consider the measurement of progesterone levels unsuitable for monitoring fetal health in the later weeks of pregnancy.

Other laboratories should takes these results as guidelines only. Because of differences which may exist between laboratories and locales in respect of population,

diet, laboratory technique and selection of reference groups. it is important for each laboratory to establish by similar means the appropriateness of adopting the normal ranges suggested by this study.

APPENDIX E

Lactic Acid Assay

(Calbiochem-La Jolla, California)

Test Principle

$$\text{L-lactate} + \text{NAD} \xrightarrow{\text{LDH}} \text{Pyruvate} + \text{NADH}$$

Pyruvate Trapping Reaction

$$\text{Pyruvate} + \text{L-glutamate} \xrightarrow{\text{GPT}} \text{L-alanine} + \alpha\text{-KG}$$

Abbreviations: α-KG = α-Ketoglutarate

GPT = Glutamate pyruvate transaminase

LDH = Lactate dehydrogenase

NAD = Nicotinamide-adenine dinucleotide

NADH = Nicotinamide-adenine dinucleotide, reduced.

In this reaction, lactate is oxidized to pyruvate and a molar equivalent of NAD is reduced. The pyruvate formed in the reaction is converted to alanine in the GPT reaction thus pulling the LDH reaction to completion. The change in absorbance at 340 nm is proportional to the concentration of lactate in the sample.

Reagent Composition

"A" vials (reagent), when reconstituted according to instructions, will yield a solution containing approximately

TRIS buffer	0.22 moles/liter
Glutamate	2.2×10^{-2} moles/liter
GPT	2400 IU/liter
LDH	21,00 IU/liter
pH 9.5	

"B" vials (cofactor), when reconstituted according to instructions, will yield approximately

NAD 3.1 x 10-3 moles/liter

Non-reactive stabilizer

U.S. Patent 3,573,171 and foreign patents.

Precautions

Vial contents may be under vacuum. If there is any indication moisture has penetrated the seal, discard the vial in question.

Reconstitution of the Reagents

A. Stat-Pack TM(Cat. No. 869218)

1. Reconstitute the contents of one of the B vials (cofactor) with 15 ml of distilled water. Cap and invert gently.

2. Open one of the A vials and transfer the entire contents of the B vial into it. Cap and invert gently.

Specimen Collection and Preparation for Analysis

1. To 2 ml of ice-cold 0.6M perchloric acid in a centrifuge tube, add 1 ml of whole blood, plasma, serum or other type of specimen. Mix well by thoroughly shaking while keeping the mouth of the tube covered with Parafilm. Let stand for 5 minutes at 3000 rpm. Use the supernatant for the assay.

Lactic acid levels in blood increase due to breakdown of glucose after the blood is drawn. If blood is cooled and spun within 15 minutes of collection, the plasma is stable at -20 C for 38 days. It has been reported that glycolysis is arrested for 24 hours at 4°C in blood collected in fluoride-oxalate(3).

Lactic acid has been reported to be stable in perchloric acid filtrates of oxalated blood for one week at 30°C(3).

Interfering Substances

A number of drugs and substances affect the rapid lactate assay. Young, et al. (4) have published a comprehensive list of such substances.

Assay

1. Using a suitable pipet, dispense 2.9 ml of the prepared reagent into a clean, dry cuvet with a 1 cm light path.
2. Using water as a blank, make all measurements at 340 nm.
3. Place the cuvet in a constant-temperature waterbath for 3 minutes or as long as needed to bring the reagent to the established temperature. Preincubate the sample for the same length of time in the same bath. Calbiochem recommends this test be performed at 30°C.
4. Wipe the cuvet dry and immediately insert into the temperature-controlled cell compartment of the photometer. A constant temperature must be maintained while obtraining assay values.
5. Measure the initial absorbance (Ao) of the reagent.
6. Add 0.100 ml (100 Ul) of sample to the cuvet. Mix quickly by gentle inversion with a square of Parafilm over the mouth of the cuvet. Avoid shaking as this can trap air bubbles in the solution. Replace the cuvet in the constant temperature waterbath. Simultaneously, start a timer.
7. After 15 minutes read the final absorbance (A15).
8. If plasma is used and the net absorbance change of the sample is greater than 1.15, repeat the test using less sample. If a protein-free filtrate is used and the net absorbance change for the sample is greater than 0.4, repeat the test using less sample. In either case, use the dilution factor D to compensate for the dilution.

Calculation

A. For Protein-free filtration:

Lactecacid concentration in mg/dL = $\Delta A \times 131 \times D$. ΔA is the observed change in absorbance after the reaction has proceeded to completion. 131 is the conversion factor. Δ is a dilution factor.

APPENDIX F

Perceived Exertion Values

Subject:

Phase of Cycle	Date	Time Wt(kg)	1 min	2 min	3 min	4 min	5 min	6 min	7 min	8 min	9 min	10 min	11 min	12 min
Day 1														
Day 1														
Midfollicular														
Midfollicular														
Midluteal														
Midluteal														

APPENDIX G

Max Run Time, Lactic Acid, Estradiol and Progesterone Values

Subject: _____

DATE	Day 1	Midfollicular	Midluteal
Max Run Time (seconds)			
Lactic Acid (m mol/L)			
1. Resting			
2. Submax			
3. Max			
Estradiol p mol/L			
1. Resting			
2. Submax			
3. Max			
Progesterone n mol/L			
1. Resting			
2. Submax			
3. Max			