

MENSTRUAL CYCLE DYSFUNCTION AND WEIGHT LOSS PRACTICES  
AMONG COLLEGE-AGE WOMEN

by

Michele D. Lewis

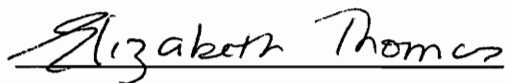
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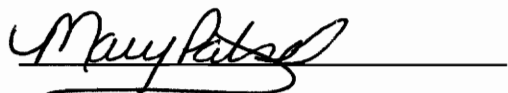
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(ABSTRACT)

Secondary amenorrhea, ovulatory disturbances, and luteal phase deficiency occur in normal-weight women with sub-clinical eating disorders. The purpose of this investigation was to examine the influence of Eating Attitudes Test (EAT-26) scores, energy intake, and frequency of activity on ovarian hormone status in normal-weight, college-age women. Fourteen normal-weight female students, ages 19 - 24, who were attempting weight loss and did not currently meet diagnostic criteria for an eating disorder, served as subjects. Food-intake, dieting behavior, and menstrual cycle function were recorded by subjects during a three month period. Biweekly blood samples were assayed for estradiol and progesterone for one menstrual cycle. Mean age, number of years dieting, and BMI were similar between subjects. Five subjects (36%) had progesterone levels indicative of luteal phase deficiency or anovulation. The EAT-26 score was not associated with menstrual cycle dysfunction. Frequency of exercise and serum progesterone concentration

were significantly correlated. Subjects who exercised 7+ hours per week had significantly lower peak progesterone values ( $p < 0.03$ ) than subjects who exercised 1 -3 hours per week. Within the group of subjects scoring above 20 on the EAT-26, those who exercised 7+ hours per week had significantly lower peak progesterone values than subjects exercising 1 - 6 hours ( $p < 0.03$ ). There was a positive correlation ( $r = 0.384$ ,  $p = 0.21$ ) between length of luteal phase and daily energy intake, however the relationship was non-significant. Normal-weight, premenopausal women dieting to lose weight experienced menstrual cycle dysfunction in the absence of significant weight loss or diagnosable eating disorder.

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## TABLE OF CONTENTS

LIST OF TABLES.....	viii
LIST OF FIGURES.....	ix
LIST OF ABBREVIATIONS.....	x
CHAPTER I        INTRODUCTION.....	1
Statement of the Problem.....	4
Research Hypotheses.....	6
Definitions.....	7
The Normal Menstrual Cycle.....	9
CHAPTER II       REVIEW OF THE LITERATURE.....	12
Introduction.....	12
The Prevalence of Eating Disorders.....	13
The Continuum of Disordered Eating.....	20
The Effects of Disordered Eating on the Menstrual Cycle.....	23
Anorexia and Bulimia Nervosa and the Sub-Clinical Eating Disorders.....	25
Dietary Restraint.....	32
Dieting and Simple Weight Loss.....	34
The Role of Body Fat in the Etiology of Menstrual Cycle Dysfunction.....	40
Effects of Athletic Training on Menstrual Hormone Status.....	43
The 26-Item Eating Attitudes Test.....	45
Physiological Consequences of Disordered Eating.....	48
Infertility.....	48
Induced Ovulation and Pregnancy Outcome.....	51
Bone Health.....	54
Summary and Purpose.....	58

CHAPTER II	METHODOLOGY.....	62
	Subjects.....	62
	Experimental Design and Methods.....	63
	Sample Collection and Hormone Assay.....	69
	Statistical Analysis.....	71
CHAPTER IV	RESULTS.....	75
	Descriptive Characteristics of Subjects.....	75
	Anthropometric Measures.....	78
	Menstrual History.....	79
	Food Intake Records.....	81
	Dieting and Activity Records.....	83
	Serum Estradiol and Progesterone and EAT-26 Scores.....	85
	Length of Luteal Phase and Daily Energy Consumption.....	89
	Serum Estradiol and Progesterone and Frequency of Exercise...	91
CHAPTER V	DISCUSSION AND CONCLUSIONS.....	96
	Introduction.....	96
	Assessment of Subject Compliance.....	97
	Factors Affecting the Menstrual Cycle.....	98
	Limitations and Suggestions for Future Research.....	110
	Conclusions.....	114
	LITERATURE CITED.....	116
	APPENDIX A.....	125
	APPENDIX B.....	127
	APPENDIX C.....	128
	APPENDIX D.....	129
	APPENDIX E.....	132

APPENDIX F.....133  
APPENDIX G.....136  
APPENDIX H.....137  
APPENDIX I.....138  
APPENDIX J.....139  
APPENDIX K.....140  
APPENDIX L.....142



## LIST OF TABLES

Table 1.	Descriptive characteristics of subjects.....	75
Table 2.	Selected methods for weight loss.....	76
Table 3.	Mean baseline and post-test weights, percent of desirable weight, and percent body fat for subjects.....	78
Table 4.	History of menstrual cycle abnormalities in SC and ND subjects.....	80
Table 5.	Low, high and mean daily energy intake among subjects.....	82
Table 6.	Reported frequency of dieting and food-related behaviors.....	84
Table 7.	Minimum, maximum, and mean serum progesterone and estradiol levels during the course of one menstrual cycle.....	85
Table 8.	Length of luteal phase and mean energy intake per day of all subjects.....	89
Table 9.	Serum progesterone and estradiol levels across differing exercise frequencies.....	93
Table 10.	Maximum and minimum serum progesterone levels in SC subjects.....	94

## LIST OF FIGURES

Figure 1.	Estradiol, progesterone, LH and FSH secretion in the normal menstrual cycle.....	11
Figure 2.	Serum estradiol and progesterone on two patients with bulimia nervosa. ....	30
Figure 3.	Time course of serum progesterone concentration during one menstrual cycle in restrained and unrestrained eaters.....	35
Figure 4.	Course of serum estradiol and progesterone in a 20 year old woman during control and diet cycles.....	38
Figure 5.	Experimental design. ....	64
Figure 6.	Serum progesterone and estradiol values across one menstrual cycle. ....	88
Figure 7.	Relationship between luteal phase length and kcals consumed per day. ....	90
Figure 8.	Serum estradiol and progesterone values for SC group according to exercise frequency. ....	95

## LIST OF ABBREVIATIONS

ANOVA	analysis of variance
BHBA	betahydroxybutyric acid
BMI	body mass index
DBW	desirable body weight
DSM	Diagnostic and Statistical Manual of Mental Disorders (III- 3rd edition; III-R- 3rd edition, revised; IV- 4th edition)
EAT-26	26-item Eating Attitudes Test
EAT-40	40-item Eating Attitudes Test
EDI	Eating Disorder Inventory
EDI-2	Eating Disorder Inventory-2 which includes the asceticism, impulse regulation and social insecurity subscales
EDNOS	Eating Disorder Not Otherwise Specified
ELISA	Enzyme-Linked Immunosorbant Assay
FSH	follicle stimulating hormone
GnRH	gonadotropin releasing hormone
IBW	ideal body weight
IRB	Institutional Review Board
LH	lutening hormone
ND	normal dieter
SC	sub-clinical
T <sub>3</sub>	triiodothyronine

## CHAPTER I

### INTRODUCTION

The co-occurrence of menstrual cycle dysfunction and the eating disorder anorexia nervosa has been widely studied (Pirke et al. 1985a, Devlin et al. 1989, Fichter and Pirke 1989, Levy et al. 1989). So dramatic is the effect of self-starvation on menstrual hormone synthesis and function that the Diagnostic and Statistics Manual (DSM) published by the American Psychiatric Association (1994) includes amenorrhea as a necessary condition for diagnosis of anorexia nervosa. The absence of menstrual flow experienced by women with anorexia nervosa is secondary to a severe loss of body weight and body fat (Frisch 1985). During the past decade, however, there has been growing evidence that weight loss or loss of body fat in themselves are not the only factors involved in inducing the onset of menstrual cycle dysfunction in women suffering from eating disorders. There have been several studies (Warren and Vande Wiele 1973, Falk and Halmi 1982) of the anorectic population in which researchers have found that the onset of amenorrhea occurs in a majority of anorectic women prior to any significant loss of body weight or body fat.

Studies have been conducted in the past decade to determine the presence of menstrual cycle abnormalities in the population of normal-

weight women diagnosed with bulimia nervosa. (Mitchell and Bantle 1983, Cantopher et al. 1988, Devlin et al. 1989, Kaye et al. 1990). It has been determined that although these bulimic women do not suffer dramatic loss of weight and body fat, as many as 50% of normal-weight bulimic women present menstrual cycle dysfunction ranging from amenorrhea to anovulation and luteal phase deficiency (Pirke, et al. 1987). Endocrinologists have yet to elucidate the mechanism by which this occurs. There is some speculation that the key may lie in previous incidents of weight loss and weight gain, or in weight cycling, experienced by many individuals with bulimia nervosa (Devlin et al. 1989). This may represent an underlying metabolic response to starvation during the binge-purge cycle or during periods of restriction commonly experienced by those with bulimia nervosa and may be the impetus behind disrupted hormonal activity in these women (Mitchell et al. 1983, Cantopher et al. 1988, Kaye et al. 1990, Schweiger et al. 1992a).

One far less studied group of individuals are women who present sub-clinical forms of the eating disorders. This population is one in which behaviors such as restricted food intake, compulsive exercise, self-induced vomiting and laxative misuse are used with a frequency which is much less than would allow them to be diagnosed with a clinical eating disorder. However, the obsessive drive for thinness, dissatisfaction with body shape

and size, and the infrequent use of compensatory behaviors have made this population a focus of concern. Recent studies (Rippon et al. 1988, Allison et al. 1988, Kreipe et al. 1989, Stewart et al. 1990) have indicated that disrupted menstrual hormone patterns in this population occur. Even simple weight loss within a range normal for height has been shown to induce abnormal menstrual hormone fluctuations in women having no apparent eating pathology (Pirke et al. 1985b, Pirke et al. 1986, Schweiger et al. 1987, Schweiger 1991).

While the fourth edition of the DSM (1994) clearly outlines the definitive criteria for diagnosis of the eating disorders anorexia and bulimia nervosa, and suggests criteria for the sub-clinical disorders, the physiological impact of disordered eating does not cease when diagnosis is not attained. As the behaviors associated with the eating disorders appear to rest on a continuum of severity, the physiological consequences of those behaviors also rest on a continuum of severity. Thus, normal weight women with the mildest forms of eating pathology or who participate in chronic dieting may experience dysfunction of menstrual hormone synthesis and activity.

## Statement of the Problem

The implication that dieting behavior can have a dramatic effect on menstrual cycle function is crucial to our understanding of fertility and the bone health of premenopausal women. As many as 18% of couples are unable to conceive in the first year (Stewart 1992). Until recently, it was assumed that among the eating disorders, only the anorectic with amenorrhea contributed to the overall rates of infertility. Overt emaciation associated with anorexia nervosa as well as obesity are difficult for clinicians to overlook. Therefore, treatment for infertility in these populations has traditionally been dietary. Currently, it is not routine to screen clients at fertility clinics for the presence of an eating pathology if overt symptomatology is not present (Bates et al. 1982, Allison et al. 1988, Stewart et al. 1990, Stewart 1992). The woman whose eating disorder remains undisclosed while ovulation is pharmacologically induced may be at risk for miscarriage or difficulties with the pregnancy and delivery (Lacey and Smith 1987, Abraham et al. 1990). There is also concern for the unborn children whose prenatal and neonatal health may be jeopardized. There is little research supporting the premise that dietary practices associated with sub-clinical eating disorders and chronic attempts at weight loss can effect menstrual cycle hormones and therefore, fertility. Further exploration into

the etiology of this dysfunction is needed so that clinicians working with this population might better educate clients about the potential physiological effects of aberrant weight loss practices. Invasive and expensive procedures used to induce ovulation, as well as the complications arising from pregnancies in women with undisclosed eating disorders, may be avoided if further dietary and behavioral links to disorders of menstrual function are discovered and appropriate screening procedures are employed routinely for women trying to become pregnant.



## Research Hypotheses

1. Normal-weight women dieting to lose weight who score 20 or above on the 26-Item Eating Attitudes Test (EAT-26), will experience a higher incidence of irregular menses, amenorrhea, oligomenorrhea, anovulation, and luteal phase deficiency than normal-weight, dieting women scoring below the symptomatic cut-off score of 20 on the EAT-26.
2. In the sample of normal-weight women dieting to lose weight, irregular menses, amenorrhea, oligomenorrhea, anovulation, or luteal phase deficiency will occur with greater frequency in women whose average daily caloric intake is at or below 1200 kcals.
3. In the sample of normal weight women dieting to lose weight, the incidence of irregular menses, amenorrhea, oligomenorrhea, anovulation, or luteal phase deficiency will be greater in women who self-report purposeful restriction of caloric intake, use of self-induced vomiting, use of pharmacological purgatives, and use of exercise to compensate for food intake and to promote weight loss.

## Definitions

Behaviors associated with eating disorders - refers to fasting, binge eating, vomiting, misuse of laxatives and/or diuretics, and excessive exercise (Garner 1991a)

Eating disorders (or disordered eating) - refers to anorexia nervosa and bulimia nervosa as defined in the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) (American Psychiatric Association (1994) (Appendix A).

Estradiol - the most potent naturally occurring human estrogen produced in the ovaries, adrenal gland, and adipose tissue (Terin et al. 1993).

Hypothalamic or Secondary amenorrhea - the cessation of menstrual periods anytime between menarche and menopause when the cause is not pregnancy. Secondary amenorrhea is associated with physiological, psychological, or nutritional stress (Speroff et al. 1978).

Irregular menses - refers to consecutive menstrual cycles of lengths varying from ten days to 40 days (Speroff et al. 1978).

Luteal Phase Deficiency - recurrent postovulatory deficiency in the production and/or effect of progesterone from the corpus luteum often leading to infertility or spontaneous abortion (Soules, 1989).

Oligomenorrhea - refers to menstrual cycles that consistently exceed 40 days (Terin et al. 1993).

Primary amenorrhea - failure to menstruate by the age of 18 years related to chromosomal abnormalities, malformation of the reproductive tract, and disorders of the thyroid, adrenal cortex, or pituitary gland (Terin et al. 1993).

Short luteal phase - refers to a variant of luteal phase deficiency in which the luteal phase is abnormally short or less than 10 days (Soules 1989).

Subclinical eating disorders (or disordered eating) - refers to the presentation of the attitudes, beliefs or behaviors associated with anorexia nervosa and bulimia nervosa without fulfilling the DSM-IV criteria for diagnosis of an eating disorder.

## The Normal Menstrual Cycle

The delicate balance of the adult female's menstrual cycle involves the close interplay between the hypothalamic-pituitary axis, the ovarian follicle, and corpus luteum. During the stages of follicular differentiation and growth, follicle stimulating hormone (FSH) and lutenizing hormone (LH) are released from the pituitary gland in response to stimulation by the hypothalamic hormone gonadotropin releasing hormone (GnRH). Lutenizing hormone activates the inactive androgen precursors of estrogen found in the ovarian follicle and in the adipose tissue of the breast, abdomen, and the fatty marrow of the long bones. The role of FSH is to facilitate the conversion of those androgens to estrogen and to promote follicular growth.

As the estrogen concentration increases during follicular differentiation and growth, a long-loop negative feedback system decreases the concentrations of FSH. At this time, a dominant follicle and oocyte are selected. A long-loop positive feedback system then responds to high concentrations of estrogen and this results in a rapid surge of LH. This is known as the midcycle gonadotropic surge and culminates in release of the oocyte and follicle from the ovary, or ovulation. After ovulation, the cells of the follicle immediately lutenize, forming a temporary secretory gland, the

corpus luteum. Stimulation of receptor cells on the corpus luteum by LH promotes *denovo* synthesis of estrogen and progesterone. Among the many functions of estrogen in the body, it is responsible for increasing the strength of uterine and extra-uterine capillary walls, promoting the growth and development of vaginal and uterine musculature, and promoting bone formation. In studies of reproductive ovarian hormone status estradiol is the most commonly assayed as it represents the most potent and abundant form of estrogen. Progesterone promotes the proliferation of the endometrial lining of the uterus preparing for the implantation of a fertilized ovum. Because progesterone is produced primarily from the corpus luteum, serum levels can be used as indicators of ovulation (Speroff et al. 1978). Rising progesterone and estrogen levels from the corpus luteum activate the hypothalamic opiate center which decreases LH and FSH secretion through decreased release of GnRH.

Degradation of the corpus luteum occurs within 12 - 15 days after ovulation. Estrogen and progesterone levels then decrease and this causes the endometrial lining to be shed. Figure 1 illustrates the biphasic and monophasic peaks of estradiol and progesterone, respectively, as well as the midcycle LH and FSH surge during the normal menstrual cycle. The typical follicular phase is 14 days and the luteal phase normally ranges from ten to 15 days (Terin et al. 1993). Metcalf et al. (1983) reported that regular ovarian

cyclicity, specifically the occurrence of regular ovulatory cycles, occurs 82% of the time at five years after menarche. Regular ovulation steadily increases to over 95% of the time by the eleventh year after menarche.

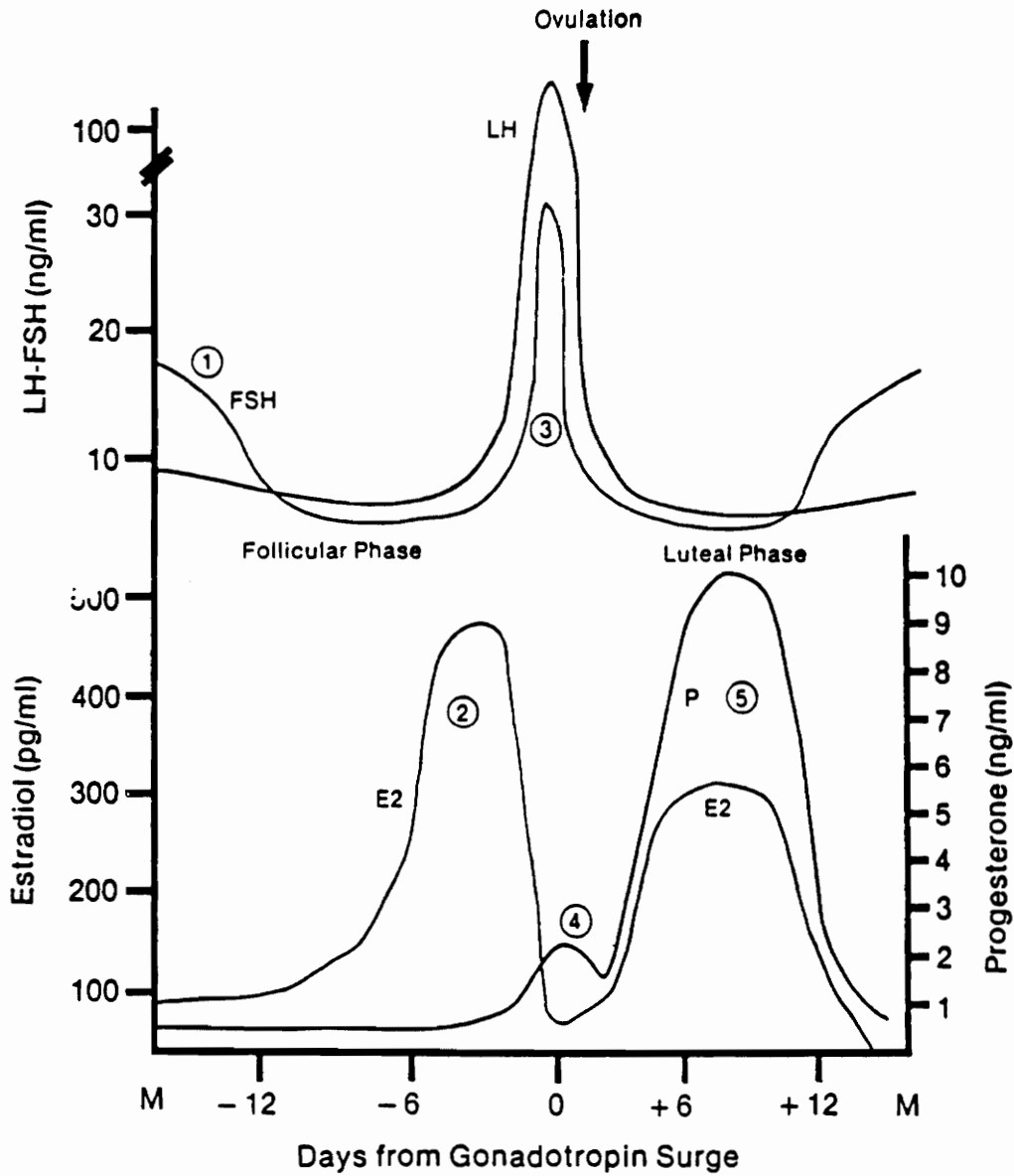


Figure 1. Estradiol, progesterone, LH, and FSH secretion in the normal menstrual cycle (Terin et al. 1993).

## CHAPTER II

### REVIEW OF THE LITERATURE

#### Introduction

It has been suggested that eating disorders, although distinct diagnostic entities according to the Diagnostic and Statistics Manual of Mental Disorders (American Psychiatric Association 1994), include a variety of attitudes and behaviors which rest upon a continuum of severity (Drewnowski et al. 1994) and that even sub-clinical manifestations of these disorders carry physiological consequences for those who participate in them (Kreipe et al. 1989). Menstrual cycle dysfunction is one physiological effect associated with both the clinical and sub-clinical forms of eating disorders.

A discussion of the prevalence of disordered eating among adolescents and college-age females and a synopsis of investigations supporting a continuum of behaviors and attitudes associated with the eating disorders begin this review of the literature. This will be followed by a review of the most recent research describing effects of disordered eating and chronic dieting for weight loss on menstrual hormone status. A survey of research which supports and dispels the idea that body fat is the primary determinant of menstrual hormone status during weight loss practices, with specific

emphasis on exercise-induced menstrual cycle abnormalities, follows. Next, the use of the 26-item Eating Attitudes Test to assess the risk of disordered eating and concomitant menstrual hormone dysfunction will be addressed. Finally, the potential effects of menstrual cycle disturbances on the fertility of premenopausal women, as well as a brief discussion of pharmacologically induced pregnancy outcome, will complete this review of the literature.

### The Prevalence of Eating Disorders

In a recent issue of the *College Student Affairs Journal*, M. Elizabeth Martin (1993) wrote “the incidence of eating disorders seems to be increasing to near epidemic proportions”. Although physicians first described the eating disorder anorexia nervosa over 300 years ago (Silverman, 1993), anorexia nervosa and bulimia nervosa, as well as the recently identified sub-clinical eating disorders, afflict a proportionately larger number of young men and women each year. Symptoms of eating disorders include compulsive binge eating, self-induced vomiting, laxative abuse, compulsive exercise, and self-starvation, coupled with a preoccupation with body size and weight (Mitchell et al. 1985).

Prevalence studies in the last decade have focused upon the secondary school and college-age populations as they seem to be at the greatest risk for



developing eating disorders (Striegel-Moore et al. 1989, Fisher et al. 1991, Whitaker 1992). Reports of the incidence of eating disorders have ranged from 1-19% of the female population in Western, industrialized countries, with sub-clinical eating disorders being the most prevalent and anorexia nervosa affecting the lowest portion of those presenting with diagnosable disorders. The primary reason for this great range of prevalence stems from the fact that researchers have adopted a variety of definitions of the eating disorders for use in investigations. Also, the methods used, self-report and interview being the most common, promote a wide range of responses depending upon the population and the means by which the assessment tools are administered. The DSM-IV has outlined definitions of the eating disorders anorexia nervosa, bulimia nervosa, and the Eating Disorders Not Otherwise Specified (EDNOS) in concise terms and is now most commonly used in research as the point of reference from which prevalence studies define the incidence of clinical eating disorders (Appendix A). While psychometric assessment tools such as the Eating Disorder Inventory (EDI) (Garner et al. 1991b) and the Eating Attitudes Test (EAT-40 and EAT-26) (Garner and Garfinkel, 1979) have been validated for use in identifying populations at risk for developing eating disorders and continue to be used in prevalence studies, the diagnosis of an eating disorder still requires a clinical interview.

Anorexia Nervosa for many decades was the most widely studied of the eating disorders. Pope et al. (1984) found a prevalence of DSM-III criteria anorexia nervosa of 1.0% and 4.2% of adolescent and college-age females (n=544) who had completed a confidential questionnaire without clinical interview. According to the American Psychiatric Association, however, current research places the prevalence of DSM-IV anorexia nervosa in the population of late adolescent and college-age women at 0.5%-1% (American Psychiatric Association 1994).

Bulimia nervosa was first identified as a pathology distinct from anorexia nervosa in the late 1970's. It was Gerald Russell in his publication entitled "Bulimia Nervosa: An Ominous Variant of Anorexia Nervosa" (Russell, 1979) who first described diagnostic criteria specific to bulimia nervosa. These criteria included the "patient suffering from powerful and intractable urges to overeat, avoiding the fattening effects of food by inducing vomiting or abusing purgatives, and having a morbid fear of becoming fat." Prevalence studies of the early to mid 1980's were completed using variations of Russell's criteria, as well as psychometric instruments such as the EDI and the EAT, and the stricter DSM-III criteria published in 1980. A wide range of rates of prevalence have thus been reported as definitions of bulimia have varied considerably (Pope et al. 1984, Whitehouse et al. 1988, Howat and Saxton 1988, Drownowski et al. 1988).

In 1981, Halmi et al. conducted a survey of 355 college students in order to determine the prevalence of DSM-defined bulimia nervosa (Halmi et al. 1981). They found that 19% of female respondents and 5% of male respondents had experienced all of the major symptoms of bulimia nervosa as outlined by the DSM-III. However, these results merely indicated the history of bulimic-like behaviors and were not based on clinical interview. Gross and Rosen (1988) found that 19.6% of females and 1.2% of males from a geographically, racially, and economically diverse sample of 1,373 high school students met DSM-III criteria for bulimia nervosa. The authors, however, based their investigation on responses to the EAT-40 and EDI subscales, assuming that student responses to these assessment tools corresponded to the DSM-defined diagnostic criteria. Schotte and Stunkard (1987) included follow-up interviews in a subsample of 1965 college-age respondents to a self-report survey which was based on the revised DSM-III. These investigators found that 1.3% of the females and 0.1% of the males met the DSM criteria for bulimia nervosa. These findings, in part because they were supported by clinical interview, closely approximate the prevalence listed in the most recently published DSM-IV (American Psychiatric Association 1994). The DSM-IV indicates that bulimia nervosa affects between 1% and 3% of adolescent and college-age females.

It was not until recently that the eating disorders not meeting the DSM diagnostic criteria for bulimia or anorexia nervosa received recognition as conditions about which professionals needed to be concerned. The overall prevalence of the EDNOS, or the sub-clinical eating disorders, in the adolescent and college-age population is unclear. In 1992, Whitaker found that 81% of the high school girls surveyed (N=2543) desired to lose weight, 63% had dieted to lose weight in the past year, 17% had used diet pills and 8% had self-induced vomiting in the last year in an attempt to lose weight. Although 16% of respondents reported a fear of gaining weight and 27% feared looking fat, only 0.3% and 4% of the females surveyed met the DSM-III for diagnosis of a lifetime prevalence of anorexia nervosa or bulimia nervosa, respectively. The need for recognition of the sub-clinical manifestations of the eating disorders was indicated in this study. Although a small percentage of respondents actually met the diagnostic standards, a relatively large percent were dieting for weight loss, often using dangerous methods to attain those goals.

In epidemiological investigations, the sub-clinical forms of the eating disorders have been assessed, most frequently, using psychometric instruments such as the 26- and 40-item EAT and the EDI-2. While neither instrument, without clinical interview, can provide an accurate diagnosis of an eating disorder, both have been successfully used to identify aberrant

beliefs, behaviors, and attitudes associated with body image and dieting practices (Gross and Rosen, 1988, Fisher et al. 1991, Koslowsky et al. 1992). In 1991, Fisher et al. used the EAT-40 to assess eating attitudes among 268 adolescent females in a suburban high school. The investigators found that 18% of respondents were identified as being at risk for having an eating disorder through scores higher than the 30 point cut-off.

The ways in which individuals with symptomatic scores on the EAT-40 who do not meet the diagnostic criteria for the eating disorders differ from individuals with diagnosed anorexia or bulimia nervosa were studied by Bunnell et al. in 1990. Of 60 referrals to a pediatric eating disorder clinic, 12 patients were diagnosed with DSM-defined anorexia nervosa, while 21 were identified as having a sub-clinical form of anorexia nervosa. Fourteen women were diagnosed with definite bulimia nervosa while eight were categorized as sub-clinical bulimic. Sub-clinical "diagnoses" were determined by a failure to meet all of the DSM-III-R criteria for the clinical eating disorders anorexia and bulimia nervosa as well as a symptomatic score on the EAT-40. A group of five individuals, although suffering from some form of an eating pathology, could not be diagnosed with either a clinical or a sub-clinical eating disorder.

In comparing demographic, clinical and psychological variables, Bunnell et al. (1990) found that there was no clear pattern of differentiation

between subjects having the clinical and sub-clinical forms of anorexia nervosa. Using these same parameters, however, there was a distinction between the clinical and sub-clinical forms of bulimia nervosa. The patients with clinical bulimia nervosa were significantly more depressed ( $p < .01$ ) as reflected by higher scores on the Beck Depression Inventory, and had significantly higher scores on the EDI subscale for Ineffectiveness ( $p < .01$ ). Bunnell et al. concluded that the DSM-III-R categories (1989) alone might exclude a great number of individuals with eating pathology who do not meet the diagnostic criteria for anorexia nervosa, especially. In response to such findings, Striegel-Moore (1989) et al. wrote:

An accurate representation of the prevalence of eating disorders as a problem among (college) students should include and distinguish between the clinical syndrome and the prevalence of various behavioral symptoms, such as binge eating, purging, and dieting.

However, if the origin of our concern with the prevalence of eating disorders goes beyond demographics, and stems, instead, from a concern for the psychological and physiological welfare of those participating in any form of disordered eating, then we must acknowledge the existence of a continuum of beliefs and behaviors.

## The Continuum of Disordered Eating

Are the clinical forms of anorexia and bulimia nervosa, extreme points on a continuum of attitudes and beliefs about body image and weight loss, or are they distinct entities not to be categorized with less severe forms of eating pathology, not to mention, simple dieting? This question was first posed by Nylander et al. in 1971 (Garner et al. 1984) when they determined that a majority of high school students surveyed in Sweden felt fat and that nearly 10% had participated in at least three anorectic behaviors in order to accomplish weight loss. Since Nylander's work, several reports have been published which have identified the incidence of anorectic and bulimic-like behaviors in adolescent and college age populations (Schotte et al. 1987, Howat and Saxton 1988, Striegel-Moore et al. 1989, Whitaker et al. 1992).

In 1981, Halmi et al. determined that 10% of college students surveyed (n=355) had participated in purging behavior such as self-induced vomiting and laxative misuse and 13% of those surveyed had at one time experienced all of the major symptoms of bulimia as outlined in the DSM-III, however, none were currently diagnosed with bulimia. The results from this study implied that the attitudes and weight loss behaviors associated with eating pathology did not necessarily fall within neat diagnostic categories.

Garner et al. (1984) conducted a study comparing the psychological

traits of a weight-preoccupied sample of college students and ballet dancers with women meeting the DSM-III diagnostic criteria for anorexia nervosa. The weight-preoccupied group, identified by high scores on the Drive for Thinness subscale on the Eating Disorder Inventory (EDI), was further divided into two groups identified by over-all high scores on all subscales of the EDI and high scores on the Drive for Thinness, Body Dissatisfaction, and the Perfectionism sub-scales only. This latter group was identified as "Normal Dieters". The Bulimia, Body Dissatisfaction, Perfectionism, and Maturity Fears subscale scores of the former weight-preoccupied group so closely emulated those of the anorectic subjects that Garner et al. (1984) concluded that food and weight preoccupation common to patients with anorexia nervosa must occur on a continuum. However, weight preoccupied subjects did not demonstrate the same psychosocial impairment found in the patients with anorexia nervosa and the authors emphasized the importance of a multidimensional evaluation of individuals suspected of having anorexia nervosa. This conclusion was underscored by the results from an investigation of purging and non-purging normal-weight bulimic females conducted by Willmuth et al. (1988). The investigators found that the purging bulimics demonstrated greater anxiety about eating, greater body size distortion and desire to be thin, and greater disturbance on a standardized



measures of eating attitudes. Therefore, even within a particular diagnosis, there existed varying levels of anxiety and psychopathology.

Finally, Drevnowski et al. (1994) published the results from a longitudinal study conducted to determine if a continuum of severity of bulimia nervosa did exist and if so, if college-age individuals, with time, moved along that continuum toward or away from more severe manifestations of pathology. The investigators developed the Eating Pathology Scale for use in classification of subjects as non-dieters, casual dieters, intensive dieters, dieters at risk, and subjects with DSM-III-R- defined bulimia nervosa. Surveys were completed in both the fall (n=902) and spring (n=704) semesters of the subjects' freshman year. The researchers received responses for both semesters from 557 women and used this data for analysis of changes in eating pathology.

Drevnowski et al. (1994) noted that shifts in severity of behavior over time occurred primarily between adjacent categories. The subjects identified in the spring semester as having bulimia nervosa had moved from the intensive dieters and dieters at risk categories into the bulimic category. Also, those subjects identified as bulimic in the fall semester, whose symptoms lessened in severity thereby moving them into a less severe category, still reported the continuation of bulimic-like behavior. Overall, 3% of the population surveyed were identified as bulimic in both the fall and spring

semesters and, as many as 86% of college age females were dieting to lose weight during the course of this study. Two limitations to this study include the lack of a clinical interview as well as the failure to identify through anthropometric measures which portion of the sample would be considered normal weight, yet still dieting to lose weight. What this study does suggest, however, is that failure to meet the diagnostic criteria for an eating disorder does not exclude an individual from having an eating pathology, nor does it prevent young women from experiencing the physiological complications associated with sub-optimal nutrition and extreme dieting behavior.

### The Effects of Disordered Eating on the Menstrual Cycle

It has been determined that eating disorders, depending upon the severity of behaviors, can result in a range of reproductive abnormalities including amenorrhea, failure to ovulate, infertility, low maternal weight gain, low birth weight, and increased neonatal morbidity (Stewart, 1992). Hypothalamic amenorrhea, or secondary amenorrhea, can result from restricted caloric intake as well as intense exercise (Pirke et al. 1985b, Kurzer and Calloway, 1986, Schweiger et al. 1987). It is thought that extreme weight loss due to inadequate energy intake, common to anorexia nervosa, causes a

cessation of GnRH secretion from the hypothalamus, a decrease in pituitary stores of both LH and FSH, and subsequently, diminished estrogen and progesterone levels. The most widely accepted explanation for this effect implicates an underlying neuroendocrine and metabolic response to starvation (Pirke et al. 1985a, Devlin et al. 1989, Fichter et al. 1990). Diet-induced secondary amenorrhea is distinguished from amenorrhea of primary origin, in which there is a congenital absence of the uterus or a deficiency of androgen receptors, and from other forms of secondary amenorrhea induced by trauma to, or surgical removal of, the viable reproductive organs.

While anorexia nervosa represents a prototype of hypothalamic amenorrhea, the behaviors associated with normal-weight bulimia nervosa, simple weight loss, and restrictive dieting can effect the secretion of lutenizing hormone (LH) from the pituitary gland (Cantopher et al. 1988, Levy et al. 1989). The FSH to LH ratio is generally decreased and estrogen and progesterone levels are, therefore, diminished. This can result in anovulation and luteal phase deficiency as evidenced by diminished progesterone secretion from the corpus luteum (Pirke et al. 1987). Luteal phase deficiency, also called a shortened or insufficient luteal phase, is thought to be caused by inadequate preovulatory follicular development and can lead to infertility and spontaneous abortion.

Intense athletic training has also been associated with menstrual abnormalities, in particular a reduced LH which results in defects in follicular growth and differentiation (Bullen et al. 1985). The etiology of disturbances of the menstrual cycle in athletes, aside from those associated with the loss of body fat in some endurance athletes, is unclear.

### Anorexia and Bulimia Nervosa and the Sub-Clinical Eating Disorders

Of the eating disorders, anorexia nervosa presents the most life-threatening physiological consequences, most of which are associated with the complex metabolic processes resulting from self-starvation. These complications include dehydration, reduced gastric motility, decreased glomerular filtration rate, diabetes insipidus, hypokalemic nephropathy, cardiac atrophy, cardiac arrhythmia, and congestive heart failure (Kaplan 1990). Menstrual cycle dysfunction, including anovulation and depressed levels of circulating gonadotropins and ovarian hormones, are so common in anorexia nervosa that amenorrhea, or loss of menstrual period for a duration of 3 months, is a necessary criterion for diagnosis of anorexia nervosa by the DSM-IV classification (American Psychiatric Association 1994).

It has long been established that in women with anorexia nervosa, secondary amenorrhea is caused by hypothalamic-pituitary dysregulation

mainly attributed to a weight deficit (Cantopher et al. 1988, Fichter and Pirke 1989). Fichter and Pirke (1989) conducted an investigation of 18 patients with anorexia nervosa whose weights ranged from 51% to 73% of ideal body weight (IBW) at first assessment. The investigators wanted to determine the 24-hour secretory LH pattern during inpatient treatment and following a modest weight gain. Blood samples were taken upon admission and after a 10% gain in IBW. All patients except two had infantile LH secretory patterns upon admission. Again, except for two patients, all developed prepubertal or adult LH secretory patterns with weight increase. Adult LH secretory patterns were achieved more rapidly in anorectic patients who gained more weight, had a shorter duration of illness, and who were younger than their peers.

While low body weight and body fat is strongly associated with the dysregulation of the hypothalamic-pituitary axis in anorexia nervosa, menstrual cycle dysfunction including amenorrhea, oligomenorrhea, and anovulation have been documented in non-obese bulimic women who are of normal or above normal weight (Mitchell and Bantle 1983, Kaye et al. 1990, Pirke et al. 1985a) Devlin et al. (1989) conducted a study involving nine patients with anorexia nervosa and 12 patients with bulimia nervosa to determine baseline patterns of LH secretion as well as LH and FSH responses to two doses of exogenous GnRH. The anorectic subjects were below 80% of IBW while the bulimic subjects fell within a range of 80% to 120% of IBW.

All of the anorectic subjects were amenorrheic while ten of the bulimic subjects were either amenorrheic or oligomenorrheic. The investigators found that there was a trend toward a lower mean 24-hour LH level in both the bulimic and anorectic subjects as compared to normal controls, however, stimulation with exogenous GnRH produced an elevated LH response in the bulimic group only. Pituitary stimulation with exogenous GnRH produced a blunted LH response in the anorectic group. The authors concluded that dysregulation of the hypothalamic-pituitary-gonadal axis in eating disorder patients could not be entirely attributed to emaciation, however, the authors did not state whether menstrual status was more greatly disrupted in the bulimic women whose weights were nearer to 80% of IBW.

Levy et al. (1989) found similar results in their investigation of ten women with bulimia nervosa and nine women with anorexia nervosa. In contrast to Devlin's (1989) study, all bulimic and control subjects were within 15% of IBW. All of the anorectics, except one, were amenorrheic and only two of the ten bulimic subjects were amenorrheic. Subjects with anorexia and bulimia nervosa had significantly lower baseline LH levels as compared to controls. All subjects received an intravenous injection of lutenizing hormone releasing hormone (LRH). Bulimic subjects had a greater peak LH and area under the LH response curve than either anorectic subjects or

controls. The investigators suggested that bulimics may maintain a sufficient pituitary reserve of LH despite low baseline LH.

Cantopher et al. (1988) assessed the frequency of menstrual disturbance in 14 normal-weight women diagnosed with bulimia nervosa. Of this sample, five subjects reported regular menses, six reported irregular menses, and three were amenorrheic. The investigators completed three serum progesterone assays from blood samples taken at two week intervals for six weeks. Progesterone values indicated that four patients had ovulated and 11 had not. Eight of the subjects who failed to ovulate underwent further study to determine serum LH and FSH hormone concentrations after intravenous injection with 100 ug of the LH releasing hormone analogue. Four patients showed a pattern consistent with hypothalamic dysfunction in which stimulation with exogenous LRH caused a surge of LH from the pituitary gland, and three showed a pattern consistent with a pituitary dysfunction in which exogenous LRH had no effect.

It was unclear how Cantopher et al. (1988) could have accurately assessed the occurrence of ovulation based on only three blood samples, as progesterone levels vary significantly from day to day and month to month within a single individual. Despite this limitation, the results suggest that menstrual cycle dysfunction does occur in bulimic women, without weight loss.

Pirke et al. (1987) obtained blood samples for estradiol and progesterone assays three times per week during a six week period from 15 patients with bulimia nervosa and ten healthy controls. The mean age and weight of patients and controls were 23.7 years, 99.5 % IBW and 26.2 years, 106.9% IBW, respectively. Of the patients, three were amenorrheic, eight were oligomenorrheic, and four had regular cycles. Continuously low estradiol values confirmed the absence of normal follicular development in seven of the bulimic patients. Compared with controls and patients with confirmed normal follicular development, however, these seven patients had a significantly lower body weight. Of the eight patients with normal follicular development, four reported regular cycles, yet only three developed normal luteal phase progesterone values. The luteal phase, as determined by plasma progesterone concentrations, was sufficient and of normal length in only one bulimic patient.

Figure 2 depicts the follicular luteal phase estradiol and progesterone secretion patterns in two patients who participated in this investigation (Pirke et al. 1987). Patient 1, who was 97% of IBW had no increase in estradiol nor progesterone during the course of her menstrual cycle. This pattern was indicative of a lack of follicular development and anovulation. Patient 2 (121% of IBW), on the other hand, had an increase in estradiol during the luteal phase, but an absence of progesterone secretion from the corpus



luteum. This second pattern depicted the luteal phase deficiency common among bulimic women.

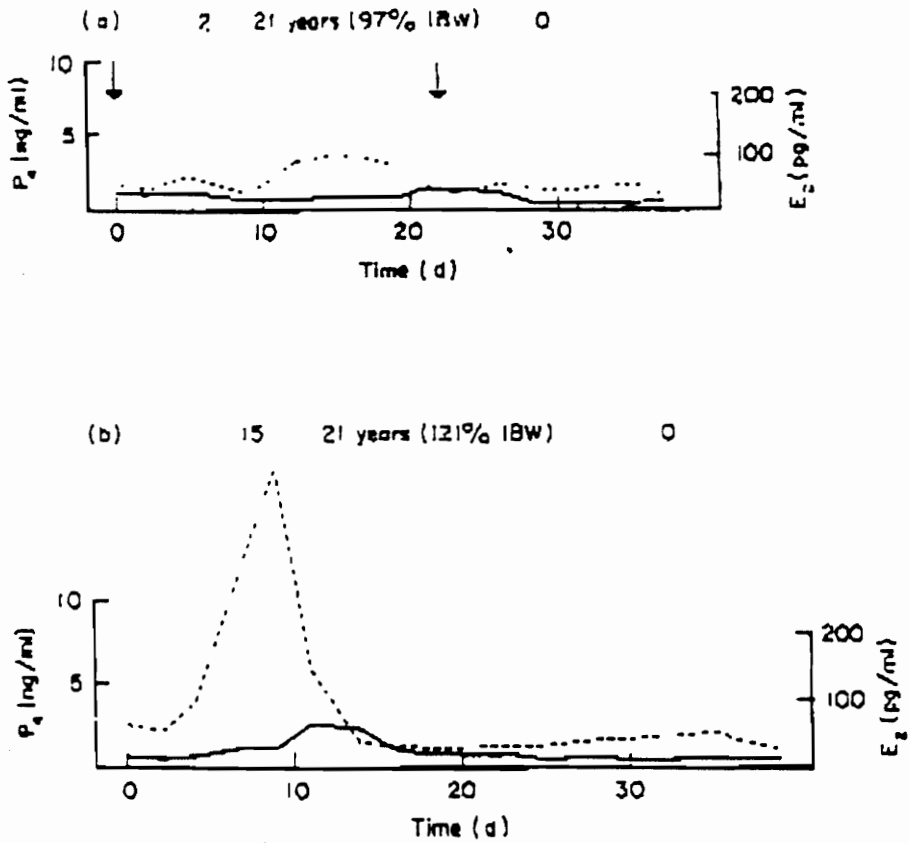


Fig. 1. (---) Oestradiol ( $E_2$ ) and (—) progesterone ( $P_4$ ) in two patients with bulimia nervosa. O, Oligomenorrhoea. In patient 2 (a) no increase of  $E_2$  greater than 120 pg/ml was observed. In patient 15 (b)  $E_2$  increased, but no progesterone increase greater than 3 ng/ml occurred.

Figure 2. Serum estradiol and progesterone in two patients with bulimia nervosa (Pirke et al. 1987).

It has been demonstrated that the patterns of aberrant weight loss behaviors evident in the clinical eating disorders also occur with less severity and frequency in sub-clinical manifestations of these disorders. If disordered eating occurs along a continuum of behaviors and beliefs, then the physiological consequences of these behaviors might also fall along a continuum of severity. The incidence of menstrual cycle abnormalities in individuals with sub-clinical eating disorders was first investigated by Kreipe et al. in 1989. Kreipe et al. (1989) used the 26-item Eating Attitudes Test (EAT-26) to screen for undiagnosed eating pathology in a sample of students from an introductory psychology class. The sample was comprised of 15 normal weight females who scored above the 20 point cutoff on the EAT-26 and who, upon clinical interview, were determined to not meet the DSM-III-R criteria for diagnosable eating disorders. Subjects and controls (n=17) completed surveys of weight history and menstrual history. Kreipe et al. (1989) found that while no participant was currently amenorrheic, 66% of the sub-clinical subjects and 0% of the controls had a history of amenorrhea. An additional 27% of the sub-clinical subjects and 11.6% of the controls had experienced oligomenorrhea. The sub-clinical subjects reported a history of significantly greater weight fluctuations than controls with all sub-clinical subjects reporting fluctuations as great as 10% of IBW and only ten of the controls

reporting such fluctuations. It is interesting to note, however, that of those ten control subjects, none had experienced menstrual cycle abnormalities.

In a second investigation, Kreipe et al. (1989) evaluated 99 upper-level psychology students for the presence of sub-clinical eating disorders using the Eating Disorder Inventory subscales. Seven subjects having scores indicative of sub-clinical eating disorders and 20 age-matched controls were selected and all subjects weighed between 90% and 110% of IBW. Results from a menstrual history questionnaire indicated that 71.4% of the sub-clinical subjects versus 10% of controls had experienced oligomenorrhea. History of amenorrhea was present in 28.6% of the sub-clinical subjects and in 5% of the controls. These studies represented the first to identify disturbances of the hypothalamic-pituitary-gonadal axis in normal-weight subjects not meeting DSM criteria for eating disorders, but whose eating attitudes represented a lesser degree of pathology.

### Dietary Restraint

While high dietary restraint, as determined by scores on the restraint scale of the Three Factor Eating Questionnaire (Stunkard and Messick 1985), is common among individuals with anorexia nervosa and in bulimic and sub-clinical cases who experience intermittent periods of severe dietary

restriction, it does not stand alone as a recognized eating disorder. None the less, the metabolic and endocrine effects of high dietary restraint are evident in a number of studies (Pirke et al. 1989b, Laessle et al. 1989, Schweiger et al. 1992). Using a model of restrained eating, Fichter and Pirke (1984) tested the hypothesis that restrictive dieting that does not result in weight loss below what is normal for height may alter reproductive endocrine function in normal-weight, young women. The researchers studied the effects of total caloric deprivation for three weeks on the secretion of LH in five female subjects. While no subject reached a weight below the range considered normal for height, after two to three weeks of fasting, three out of five of the subjects showed LH secretion pattern regression to infantile levels. All subjects were amenorrheic for the duration of the study and for at least six weeks following the study. The results of this study indicated that severe caloric restriction can induce menstrual cycle abnormalities in women with previously normal cycles.

Schweiger et al. (1992b) identified subjects with high cognitive restraint using the Three Factor Eating Questionnaire's restraint scale. Women having scores above the 75th percentile on the restraint scale were classified as restrained eaters. Women with scores below the 50th percentile were classified as unrestrained eaters. Thirteen unrestrained eaters and nine restrained eaters, ages 19-24, provided daily blood samples for progesterone

and estradiol assay over the course of one menstrual cycle. No group differences with respect to age, BMI, height or weight were detected. The average energy intake during the first week of the cycle was significantly higher ( $p < 0.05$ ) in the unrestrained group ( $2170 \pm 521$  kcal) as compared with the restrained group ( $1724 \pm 439$ ), but did not significantly differ during the remainder of the study. Eleven unrestrained subjects had menstrual cycles which met the standard criteria for estradiol maximum and progesterone maximum, however, only two of nine of the restrained subjects cycles met these criteria. The most significant difference between groups was the lower luteal phase progesterone concentrations in the restrained eaters, which was indicative of a luteal phase deficiency and anovulation. Figure 3 provides an illustration of luteal phase progesterone values in these restrained and unrestrained eaters. While the time course of serum progesterone differed among subjects, the absence of a luteal phase peak, as well as luteal phase progesterone values below 3 ng/ml determined luteal insufficiency (Schweiger et al. 1992). This study indicated that ovarian function may be highly sensitive to restrictions of caloric intake, even when caloric intake did not appear especially deficient to meet energy and nutrient needs.

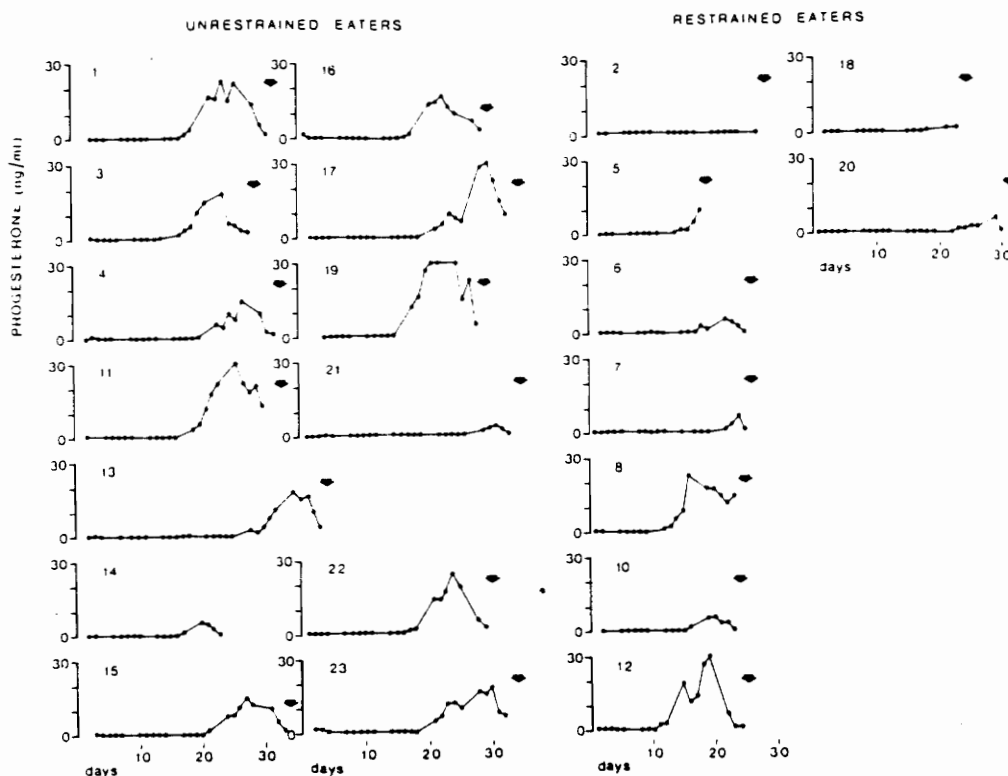


Figure 3. Time course of serum progesterone concentration during one menstrual cycle in restrained and unrestrained eaters (Schweiger et al. 1992b).

### Dieting and Simple Weight Loss

Until very recently, despite the frequency of dieting in normal-weight young women, very little was known about the consequences of dieting and simple weight loss on reproductive function. Weight loss as low as one kilogram per week, and within 10% of IBW, over the course of one menstrual cycle can lead to anovulatory cycles and luteal deficits in normal-weight women (Schweiger et al. 1987). Whitaker (1992) found that the

relative risk of developing secondary amenorrhea in girls who fasted for three or more days was significantly higher than in girls who had dieted, without fasting, for 28 consecutive days or more. Dieting females however, were not at greater risk for developing secondary amenorrhea than non-dieting females. In contrast to the duration of dieting for weight loss, other methods of weight control such as frequent self-induced vomiting, laxative use, and the use of diet pills were each associated with a one and one half to threefold increase in risk for developing secondary amenorrhea. These results indicate, again, that restrictive dieting may lead to menstrual abnormalities, however body weight and body fat were not considered in Whitaker's investigation and may have accounted for some of the differences in hormone status.

Vegetarian diets, which tend to be low in fat and low in kilocalories, are adopted by many young women in their effort to lose weight. Pirke et al. (1986) compared the effects of a 1000 kcal vegetarian, high carbohydrate diet with a 1000 kcal, high animal protein diet on the menstrual hormone status of two groups of nine healthy, normal-weight women. Blood was drawn three times per week for six weeks and all subjects had normal ovulatory cycles at baseline. Both groups lost an average of one kilogram of body weight per week. In the vegetarian group, seven out of nine subjects became anovulatory, average LH values were significantly decreased during the

midcycle and luteal phases, and luteal phase progesterone and estradiol values were also significantly lower than in the non-vegetarian group. Two out of nine subjects on the non-vegetarian diet became anovulatory, however, there was no significant reduction in LH, estradiol, or progesterone during any phase of the menstrual cycle in this group. One limitation to this study was that the physical activity of the subjects was not assessed and may have affected differences in hormone values between groups, although weight loss was identical between groups. The results of this study, however, indicated that the nutrient quality of the diet, as well as the total energy level, may significantly effect the hypothalamic-pituitary-gonadal axis during weight loss practices.

In a second study conducted by Pirke et al. (1989a), 13 healthy, normal-weight women were studied throughout a control cycle and a diet cycle, during which time they lost one kilogram of body weight per week on an 800 kcal lacto-vegetarian diet. The body mass index of subjects at the end of the dieting period was equivalent to 99% of IBW. Blood samples were assayed for LH, FSH, progesterone, and estradiol daily for two menstrual cycles and follicular development was determined by ultrasonography. All subjects demonstrated normal ovulatory cycles during the control cycle, however, follicular development was impaired in seven subjects during the diet cycle. Four subjects showed normal follicular development but impaired



progesterone secretion by the corpus luteum during the diet cycle. Lutenizing hormone secretion was impaired during dieting with average LH concentrations significantly reduced during the follicular phase only. From this investigation, it was unclear whether the menstrual cycle abnormalities resulted from caloric or nutrient deficiencies, however, the results from their previous investigation indicated that the effect may be compounded in women who practice restrictive dieting.

Figure 4 illustrates the course of estradiol and progesterone secretion in a 20 year old female subject during the control and diet cycles of a study conducted by Schweiger et al. (1987). During the diet cycle, in which subjects consumed a 1000 kilocalorie vegetarian diet, both estradiol and progesterone secretion were diminished in 14 of 22 subjects. Estradiol values were not so low as to indicate a lack of follicular development, however, luteal deficiency was evident.

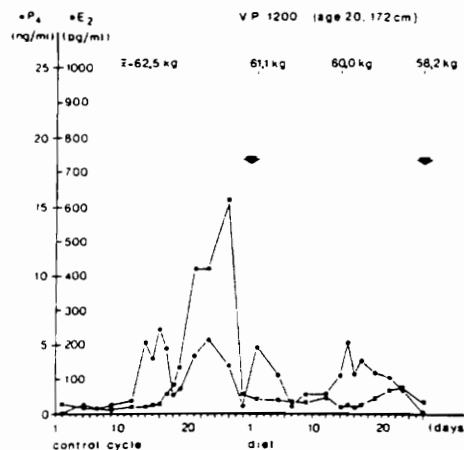


Figure 4. Course of serum estradiol and progesterone in a 20 year old woman during control and diet cycles (Schweiger et al. 1987).

The fact that weight loss diets which employ caloric deprivation lead to an increase in certain metabolic indices of starvation may be an important determinant in identifying a centrally mediated effect on menstrual cycle function. The metabolic and endocrine effects of intermittent restrictive dieting in a sample of women diagnosed with bulimia nervosa were studied by Pirke et al. (1985). The investigators found that in 15 bulimic patients, whose weights ranged from 81% to 109% of IBW, there occurred at baseline low plasma glucose, elevated free fatty acids, high betahydroxybutyric acid and acetoacetate, and low norepinephrine, all indicators of a low energy intake, at the time of the study. Similar results were obtained by Fichter et al. (1990). Twenty four women diagnosed with bulimia nervosa (from 84% to 113% IBW) and 24 normal control subjects were assessed for disturbances in the hypothalamic-pituitary-adrenal axis and other endocrine axes. Plasma FSH and LH values were significantly reduced in bulimic subjects, and the indicators of reduced caloric intake, namely, elevated betahydroxybutyric acid and low triiodothyronine, were also present.

In a 1991 report describing menstrual function and luteal phase deficiency in relation to dieting, Schweiger suggested that the short term and long term metabolic adaptations to starvation and semi-starvation have been shown to alter the function of certain neurotransmitters, like norepinephrine and serotonin, which play a role in the regulation of GnRH secretion from

the hypothalamus. Schweiger (1991) also suggested that because noradrenergic fibers and insulin receptors are found in the ovaries, decreased food intake may directly impact ovarian function through impaired glucose uptake or impaired norepinephrine secretion. However, these hypotheses remain unsubstantiated and require further investigation.

### The Role of Body Fat in the Etiology of Menstrual Cycle Dysfunction

An explanation of the etiology of hypothalamic or secondary amenorrhea associated with weight loss was first proposed by Frisch and MacArthur in 1974. According to Frisch and MacArthur (1974), a 17% body fat was the minimum required for initiating menstrual cycles at menarche, and a 22% body fat was required to sustain normal menstrual cycles. After the onset of regular menstrual cycles, a weight loss of 10-15% of normal weight for height represented a loss of body fat below the 22% level and could result in amenorrhea. The rationale behind this theory was threefold: That androgens produced by the ovaries are chemically converted to estrogen in the fatty tissue of the breast and abdomen and in the fatty marrow of the long bones; That lean women produce more of a less "potent" form of estrogen; And, that adipose tissue stores steroid hormones which are converted to estrogen. Frisch (1985) later added to this theory by stating that obese women,

who also experience menstrual dysregulation, have a diminished binding capacity for estrogen to the serum sex hormone binding globulin (SHBG). Sex hormone binding globulin regulates the availability of estrogen to the brain and other target tissues, thereby influencing the efficiency of the long- and short-loop estrogen feedback systems.

Despite this rationale, there is evidence, which suggests that body weight or body fatness are not the sole determining factors in the regulation of normal menstrual cycles. Schweiger et al. (1989b) reported that a substantial portion of anorectic patients do not resume normal menstrual function within one year after weight gain. Whittaker et al. (1992) also stated that in 16% of all anorexia nervosa cases, amenorrhea actually precedes significant weight loss.

To determine if a low body weight was the determining factor in menstrual abnormalities in patients with eating disorders, Weltman et al. (1990) compared the menstrual history of 16, normal-weight (mean  $103\% \pm 6\%$  IBW) patients with bulimia nervosa with that of 29 underweight (mean  $88\% \pm 13\%$  IBW) patients with cystic fibrosis. Of the patients with bulimia 73% had a history of secondary amenorrhea compared with 28% of those with cystic fibrosis. At the time of the study, 40% of the bulimic patients and 78% of the cystic fibrosis patients were normally menstruating. While the menstrual age, or time since menarche, was the same between groups, the

bulimic group was significantly younger and had begun menarche at an earlier age than the cystic fibrosis group. The authors did not indicate whether menarchial age could have had any confounding effect on the results that were obtained.

In a second study (1990), Weltman et al. compared the menstrual histories of 18 age and weight-matched cystic fibrosis patients with 18 anorectics. The researchers found that 100% of anorectics had experienced secondary amenorrhea while only 33% of the cystic fibrosis patients had. One patient with anorexia nervosa was having normal cycles at the time of the investigation while 14 cystic fibrosis patients currently had normal cycles. These results indicated that perhaps differences in psychological stress levels, which were not measured, or specific nutrient deficiencies might have accounted for differences, but body weight per se was not the major factor responsible for menstrual abnormalities. Dietary records were not kept during the course of either study by Weltman et al. (1990), but the authors presumed that cystic fibrosis patients were closely monitored for nutrient intake and were receiving vitamin supplementation, whereas the patients with eating disorders could not be assumed to follow prescribed diets. The researchers also suggested that menstrual dysfunction in the normal weight bulimic patient may have been due to frequent weight fluctuations caused by intermittent restrictive dieting, but because they failed to account for this

through dietary or weight history records, or through biochemical indices of semi-starvation, the precise etiology of menstrual dysfunction could not be determined.

### Effects of Athletic Training on Menstrual Hormone Status

In addition to the loss of body weight and body fat experienced by some individuals with eating disorders and the still unexplained menstrual hormone dysregulation in normal weight women, the rise in the lean body mass and loss of body fat that accompanies serious athletic training can also induce metabolic and hormonal changes. Alterations such as diminished plasma insulin, and an increased secretion of corticosteroids and catecholamines preceding competition, may also affect the regulation of menstrual cyclicity in very active women through central nervous signals to the hypothalamus (Bonen 1986).

In a study of cyclic ovarian function in recreational runners, Broocks et al. (1990) found that only ten out of 17 normal-weight athletic subjects (n=18), who normally ran 20 - 30 kilometers per week, had normal plasma progesterone and estradiol levels. All athletes had significantly lower estradiol values in all phases of the menstrual cycle when compared with normal-weight, sedentary controls (n=13), of which 11 had normal cycles.

Broocks et al. surmised that the caloric intake may have been insufficient to meet the energy needs of the athletes, however both eumenorrheic athletes and athletes with menstrual disturbances consumed similar caloric intakes.

As dieting for weight loss often involves periods of restrictive dieting, it may also involve intermittent bouts of strenuous exercise in previously untrained women. Bullen et al. (1985) studied the effects of strenuous exercise on the induction of menstrual disorders in two groups of women who had not previously participated in strenuous activity. After a preliminary five month observation period and a four week control period, during which time subjects participated in normal activities, both groups were required to run four miles per day, progressing to ten miles by the fifth week of the experimental period and to engage in three and one half hours of moderate intensity sports activities daily. Group I (n=12), the weight maintenance group, consumed a diet consisting of 50% carbohydrate, 35% fat, and 15% protein during the control and exercise periods, with adequate energy to meet the needs of increased activity during the exercise periods. Weight loss in Group I over the course of five weeks was  $1 \pm 0.2$  kilogram. Group II (n=16), the weight loss group, consumed a diet with the same ratio of carbohydrates, fats and protein and same caloric content as Group I during the control period, but with an energy content which allowed for a weight loss of  $4 \pm 0.3$  kilograms during the five week exercise period.

Upon analysis of menstrual records, serum progesterone, and LH levels, the investigators found that only four of the 28 subjects had normal cycles during the exercise period. Of those four, three occurred in the weight maintenance group. Menstrual disturbances included abnormal bleeding, delayed onset of menses after training, abnormal luteal function, and diminished peak LH. Delayed menses and diminished peak LH occurred more often ( $p < 0.05$ ) in the weight loss group. The results of this study indicated that strenuous training, even without significant weight loss, may alter the normal function of the hypothalamic-pituitary-gonadal axis. These effects occurred with and without an energy deficit. One limitation to this study was that the authors did not indicate shifts in lean body mass and percent body fat between groups and only reported a baseline percent body fat for all subjects. None the less, these results do indicate the presence of endocrine abnormalities in untrained, healthy, normal-weight women who engage in strenuous activity and who do not undergo a significant loss of body weight.

### The 26-Item Eating Attitudes Test

The Eating Attitudes Test (EAT) was developed by Garner and Garfinkel in 1979 and was originally used to diagnose anorexia nervosa in



epidemiological studies. Scores above the cut-off of 29 points on the original 40-item version and 19 points on the 26-item version of the EAT are considered indicative of an individual at risk of having or developing an eating disorder (Szmukler 1989). While the EAT effectively measures attitudes commonly associated with both anorexia and bulimia nervosa, the diagnosis of these eating disorders requires the use of a clinical interview.

In 1982, Garner et al. derived the shorter 26-item EAT from a factor analysis of the original 40-item test (Garner et al. 1982). The three factors comprising the 26-item EAT are Dieting, Bulimia and Food Preoccupation, and Oral Control. Inter-correlations between the EAT-26 and the EAT-40 indicated that total scores on the EAT-26 were highly predictive of the total scores on the EAT-40 ( $r=0.98$ ). The factor structure and criterion validity of the EAT-26 were more recently studied by Koslowsky et al. (1992). The EAT-26, as well as a five-item scale for calculating body image and a diet and weight history, were completed by 809 female soldiers. Results indicated that scores on the EAT-26 were significantly correlated with body image, weight, and number of diets in the past year. Similar results were obtained by Scheinberg et al. (1993) who found that although the EAT-26 did poorly in identifying the most extreme cases of eating disorders among a group of 231 female soldiers, it was a better predictor of the milder cases, or sub-clinical

cases, in whom only one or two of the DSM-III-R symptoms for anorexia nervosa were present.

An inherent problem with the use of any self-report questionnaire, such as the EAT, is the risk of dishonest or inaccurate responses. Denial of symptoms in a group of 66 patients with DSM-defined anorexia and bulimia nervosa was studied by Newton et al. (1988). Of the female subjects, 19.7% scored below the cut-off for either the EAT-40 or EAT-26. Low scorers were more likely to be diagnosed with anorexia nervosa and tended to score significantly lower on other measures of eating pathology such as the Body Dissatisfaction scale of the EDI. This study underscored the importance of combining clinical interview with self-report assessment tools in determining the extent of eating pathology.

Studies of menstrual dysfunction in EAT-26-defined sub-clinical eating disorders are extremely limited. Rippon et al. (1988) examined the relationship between elevated scores on the EAT-26 and menstrual dysfunction in 88 predominantly lean female ultra-marathon runners, ballet dancers, and fashion models. The subjects were categorized according to their weight classification and exercise status into low body mass exercisers, low body mass non-exercisers, and moderate body mass exercisers. The investigators found that menstrual cycle dysfunction in all groups was equally common (43-55%) and that the incidence of symptomatic EAT-26

scores were 15% in the low body mass exercisers, 39% in the low body mass non-exercisers, and 44% in the moderate mass exercisers. Elevated EAT-26 scores were strongly associated with menstrual cycle dysfunction ( $p < 0.01$ ) in all groups, however, they were not significantly associated with body mass index nor level of exercise. Of subjects with EAT-26 scores less than 20 points, 38% had irregular menses while 71% of high scorers experienced menstrual irregularity. While the results of this study seem to suggest that eating attitudes were more indicative of menstrual cycle dysfunction in lean females than body mass index or exercise intensity, the results relied completely on self-reported menstrual histories; Serum hormone levels were not directly measured. Nutrient and energy intake were not determined and could have been used to rule out the influence of dietary restriction on menstrual hormone status on some subjects.

### Physiological Consequences of Disordered Eating

#### Infertility

In 1992, Stewart reported that an estimated 15-18% of all couples in the childbearing years are unable to conceive in the first year. While the literature clearly illustrates the impact the continuum of disordered eating has on the hypothalamic-pituitary-gonadal axis, it is not infrequent that a

gynecologist will evaluate and pharmacologically manage an infertility problem due to low levels of gonadotropins or ovarian hormones and not be aware of an existent or developing eating disorder. In Whitaker's (1992) epidemiological study of the rates of anorectic and bulimic-like behaviors in adolescent females, less than one third of those students with DSM-III defined bulimia nervosa had ever discussed their problems with a professional.

Recent studies have shown that unexplained infertility in women may be due to an undisclosed eating disorder or to extreme attempts at weight control. Bates et al. (1982), conducted an intervention study of 47 underweight women who practiced weight control by caloric restriction and who presented with unexplained infertility or menstrual dysfunction at a reproductive endocrinology clinic. When 36 subjects followed a dietary regimen designed to increase their body weight to a predicted ideal body weight, 73% were able to conceive and 90% with secondary amenorrhea resumed menstruation. Bates reported that 97% of these subjects had been previously evaluated to determine the source of their infertility and were given no indication that their weight loss practices may have been the cause.

Stewart et al. (1990) reported a 16.7% prevalence rate of clinical and sub-clinical eating disorders in 66 consecutive patients at a fertility clinic. The mean age of the subjects was 30.4 years and mean weight was 103% of IBW. The EAT-26 was used in conjunction with DSM-III-R-based clinical

interviews to ascertain accurate diagnoses. Twelve of 64 patients who took the EAT-26 scored in the symptomatic range. The two patients who did not complete the EAT-26 were currently being treated for anorexia nervosa. Of the infertile women who presented specifically with amenorrhea and oligomenorrhea, 58% had DSM-defined eating disorders and none of these subjects had disclosed their eating disorder to their fertility specialist. A 7.6% prevalence of DSM-defined eating disorders in this sample of infertile women was two to four times that predicted in the population of adolescent and college-age females. These results indicated that among women with infertility problems, there may have been underlying dysfunctional eating attitudes and aberrant weight loss practices.

Allison et al. (1988) also studied female infertility patients to determine the relationship between weight, abnormal eating attitudes, symptomatic distress, and impaired ovulatory function. Those subjects with previously diagnosed anovulatory menstrual cycles (n=12) were compared with subjects who had regular ovulatory cycles (n=18). Investigators administered the EDI and the General Health Questionnaire to 30 patients, ages 23-37 years (mean 29.8 years) and with weights ranging from 70.5% - 193.5% of IBW, (mean 102%). All subjects were experiencing some degree of menstrual disturbance ranging from irregular or prolonged cycles to amenorrhea.

The anovulatory subjects were found to be more preoccupied with weight and dieting, as determined by significantly higher scores on the Drive for Thinness subscale of the EDI, and there was a nonsignificant tendency for the anovulatory women to be overweight (>110% IBW) or underweight (<90% IBW) as compared with the ovulatory subjects. These studies suggest that using the EDI and the EAT-26 as standard screening instruments in reproductive endocrinology clinics provides an important opportunity for primary prevention.

#### Induced Ovulation and Pregnancy Outcome

The risk to both mother and fetus increases with pharmacologically induced ovulation. When this is coupled with an underlying eating disorder, which may be exacerbated with maternal weight gain, the effects may be dangerously amplified. Abraham et al. (1990) found that 13 of 14 consecutive women, who had ovulation induced by GnRH-a injection, had previously met the DSM-III-R criteria for an eating disorder. Seven of these women currently fulfilled the criteria for EDNOS. The investigators reported that while 12 of these subjects successfully conceived, three subjects had experienced two spontaneous abortions each. Of the 12 live births, four infants weight less than 2500 grams and one of these infants died in the

neonatal period. While the researchers did not report the incidence of continued disordered eating during pregnancy nor the maternal weight gain before and during pregnancy, the results indicate a very high rate of morbidity among infants born to women with a history of disordered eating who had ovulation pharmacologically induced.

Lacey and Smith (1987) examined the impact of pregnancy on the eating behavior of 20 untreated normal-weight bulimics through a questionnaire and personal interview. The investigators reported that the prevalence of binge eating and self-induced vomiting decreased sequentially after each trimester and by the third trimester, 75% of subjects had completely stopped bulimic behavior. Of the 22 live births, one infant was born prematurely while the others born at term were between 2500 and 3700 grams in weight. The effects of a history of disordered eating on fetal development did not appear as severe in this study as those reported by Abraham et al. (1990). The key may have been in a reduction of pathological behaviors with increased gestation in this second group. Lacey and Smith (1987) gave no indication about the prevalence of previous infertility in their subjects, therefore, the two groups may have differed considerably.

Stewart et al. (1987) demonstrated that women in whom eating disorders were in remission at conception had greater maternal weight gain and babies with higher birth weights and five minute APGAR scores than

women who conceived while actively participating in restricting anorexia or bulimia. In contrast to the findings of Lacey and Smith (1987), Stewart et al. (1987) found that women who had the symptoms of eating disorders at conception had a continuance or worsening of these symptoms during pregnancy and in the postpartum year. Of 23 pregnancies in this investigation, 20 resulted in live birth. Low maternal weight gain before pregnancy and poor pregnancy weight gain was significantly related to an increase in intrauterine growth retardation, low birth weight, congenital abnormalities, and perinatal mortality.

Finally, the relative risk of low birth weight babies was investigated by Van Der Spuy et al. (1988). The researchers compared the birth outcomes of 41 normal and underweight pregnant women in whom ovulation was pharmacologically induced with the birth outcomes of 1212 normal and underweight pregnant women in whom ovulation was spontaneous. Normal-weight women who had ovulated spontaneously had the lowest incident (6%) of infants born small for gestational age, followed by underweight women who ovulated spontaneously (18%). Pharmacological induction of ovulation in normal-weight and underweight women produced the greatest incidence of small for gestational age infants (25% and 54%, respectively). These studies indicate that the risk of morbidity increases with the pharmacological induction of ovulation and with the continuation of



disordered eating at conception and throughout pregnancy. Identification of eating pathology and aberrant weight loss practices in women with unexplained infertility and subsequent intervention may significantly reduce the necessity of using invasive and expensive procedures to promote fertility and, more importantly, may reduce the health risk to mother and fetus.

### Menstrual Disturbances and Bone Health

Although the incidence of early-onset osteoporosis in women who practice aberrant weight loss methods is not the focus of this thesis, it is important to mention, in brief, two studies which further substantiate the need for intervention in the sub-clinical population. This importance rests in the fact that while menstrual cycle disturbances have been identified in both the clinical and sub-clinical eating disorders, bone mineral density has been shown to decrease prematurely in women who suffer from menstrual or ovulatory disturbances. This is due in part to the close dependence of bone mineral metabolism on estrogen synthesis in premenopausal women. This would imply that the mechanism by which bone is lost in this latter group may also occur in women who suffer from sub-clinical manifestations of the eating disorders, thus predisposing this population to complications arising from early-onset osteoporosis.

Joyce et al. (1990) investigated bone density in a sample of 33 female patients with eating disorders. Eight patients were diagnosed with anorexia nervosa, 17 with bulimia nervosa, and eight had sub-clinical manifestations of the eating disorders, or EDNOS. Subjects were evaluated by bone densitometry at the radius, femur and spine to determine the prevalence and distribution of osteoporosis and the role of exercise frequency and plasma estradiol levels, as well as age, length of illness, and history of amenorrhea on bone mineral status. Results indicated that the bone density was the least in all sites in the EDNOS group, with the anorectic measures being intermediate, and the bulimic group having an overall higher bone density. Age and length of illness was weakly correlated with femur and spinal bone density in the bulimic and EDNOS groups, however, exercise frequency was significantly correlated with femur bone density in the anorectic group only. The investigators reported that there was no significant correlation between bone density measurements and estradiol levels or history of amenorrhea in any group. These results indicated that estrogen deficiency alone may not have been the primary cause of premature bone loss in patients with eating disorders. However, serum estradiol levels were unavailable for an undisclosed portion of the subjects, and amenorrhea in these subjects was inaccurately assumed equivocal to estradiol deficiency.

A second study by Prior et al. (1990) offered a more complete analysis of menstrual hormone status as it related to losses in bone density. Prior et al. conducted a study of bone loss in premenopausal women who were either normally active or marathon runners and who all experienced regular, ovulatory cycles for two months prior to the study period. Sixty six women participated in the study and of these, 21 began training for a marathon, 22 ran regularly, but less intensively, and 23 had normal levels of activity. The researchers measured the menstrual cycle length, length of luteal phases, and assessed diet, exercise, and hormone levels for a period of 12 months. Bone mineral density, specifically the cancellous spinal bone from the twelfth thoracic vertebra to the third lumbar vertebra, was measured twice in the 12 month study period by quantitative computer tomography.

Ovulatory disturbances were found to occur in 29% of all subjects with no incidence of amenorrhea (defined in this study as an absence of menses for 6 or more months) occurring during the course of the study. Mean spinal bone loss for all subjects was 2% ( $3.0 \pm 4.8$  mg/cm<sup>3</sup>/yr). Bone loss was strongly associated with ovulatory disturbance ( $r=0.54$ , 24% of variance). It was noted that the women training for the marathon had cycles similar to subjects in other groups and that menstrual cycle disturbances seemed equally distributed across all groups. The 13 women with anovulatory cycles lost bone mineral at the highest rate of  $6.4 \pm 3.8$  mg/cm<sup>3</sup>/yr (4.2%). The 13 women

who maintained normal menstrual cycles throughout the study tended to show an increase in spinal bone density during the study period, while the 28 women who experienced one or more shortened luteal phases and the 13 women with one or more anovulatory cycles had significant decreases in spinal bone density. According to a multiple regression analysis, the shortened luteal phase was the strongest explanatory variable for change in spinal bone density, with caloric intake and family history of osteoporosis contributing about 4%. Finally, in 19 women who had an increase in spinal bone mineral density, there was a higher mean estimated serum progesterone level in the luteal phase as compared with the 47 women who had a decrease in spinal bone mineral density. Serum estradiol levels did not differ between subject groups.

An interesting conclusion made by Prior et al. (1990) was that an estrogen deficiency may not have been the only factor leading to bone loss in these premenopausal women. Progesterone, also known to be active in bone metabolism, appears to promote bone formation and accelerate remodeling. A deficiency in progesterone, as indicated by anovulatory cycles and shortened luteal phases, may play a greater role in bone loss that previously suspected.

## Summary and Purpose

It has been suggested that the behaviors and attitudes associated with disordered eating in the adolescent and college age populations rest on a continuum of severity, with clinical anorexia and bulimia nervosa at one extreme and restrictive dieting at the other. In a culture which equates a lean body with social success and self-worth, it is no wonder that the incidence of dieting for weight loss is as high as 81% in high school females (Whitaker 1990) and 86% in college age females (Drewnowski 1994). These figures include those who seek weight loss even when they are considered to be of normal weight. While many dispute the existence of such a continuum, the physiological consequences of disordered eating and restrictive dieting illustrate clearly that specific diagnostic criteria cannot delineate who will suffer the repercussions of extreme weight loss practices and who will not.

Menstrual cycle dysfunction has been demonstrated along the continuum of disordered eating from the emaciated anorectic to the normal-weight woman who intermittently restricts her dietary intake (Fichter and Pirke 1989, Devlin et al. 1989, Schweiger et al. 1992). The severity of this dysregulation seems to correspond to the severity of the behaviors associated with the eating disorder. Women with anorexia nervosa, bulimia nervosa, the sub-clinical forms of eating disorders, as well as restrictive dieters have

been plagued with amenorrhea, oligomenorrhea, luteal phase deficiency, and anovulation. While the etiology of these disorders in anorexia nervosa was once believed to be solely associated with the loss of body weight and body fat, the fact that normal-weight women experience these same disorders of menstrual function has called this theory into question (Schweiger et al. 1989, Weltman et al. 1990). This is an important and timely realization as normal-weight, infertile women with undisclosed eating disorders continue to have weight loss practices overlooked due to a lack of effective screening methods at reproductive endocrinology clinics (Bates et al. 1982, Stewart et al. 1990).

The group of women who have no diagnosable eating disorder, yet continue to diet for weight loss, despite being of normal-weight, is the focus of this research project. The continuum hypothesis would suggest that this group may also experience menstrual cycle abnormalities in the presence, or even the absence, of attitudes and behaviors associated with the eating disorders. Previous research has been limited in this area. Studies conducted by Schweiger et al. (1992) and Fichter et al. (1984) looked specifically at dietary restraint, without taking into consideration other dieting behaviors associated with eating pathology. Investigations which focused on the effects of simple weight loss on menstrual hormone status (Pirke et al. 1986, Pirke et al. 1989) involved the induction of menstrual dysfunction through restricting the dietary intake of normal subjects

Two studies conducted by Kreipe et al. (1989) and Rippon et al. (1988) represent the only investigations of menstrual hormone dysregulation in women scoring in the sub-clinical range on the EAT-26. In both cases, the results were based on a retrospective recall of menstrual history and weight history, instead of on specific serum hormone assays and the assessment of current dietary practices. Although the Kreipe (1989) investigation involved a relatively small sample size, body weight, percent body fat, activity level, dietary intake, and an analysis of specific weight loss behaviors were not included in the study design. Rippon et al. (1988) did include an analysis of body mass and activity levels as the means by which their subjects were divided for analysis. However, these investigators also relied completely on menstrual history, and, because they did not use diet records, the possibility of a restricted energy intake in certain subjects was not assessed.

The purpose of this investigation is to identify the effects of eating attitudes, caloric intake, and activity level on cyclic estradiol and progesterone levels in a sample of normal-weight college women dieting to lose weight. A distinction will be made between those with apparent eating pathology as determined by high scores on the EAT-26 and those who simply wish to lose weight but who do not hold attitudes or beliefs consistent with the eating disorders. The importance of this work lies not only in supporting previous research which has identified the presence of menstrual cycle dysfunction in

this population, but also it is to promote further investigation toward uncovering the etiology of these still unexplained problems. Most importantly, it is to bring to light the importance of screening infertility patients for extreme dieting practices and eating pathology, even in the absence of significant weight loss.



## CHAPTER III

### METHODOLOGY

#### Subjects

Fourteen female subjects were selected from women who responded to a flyer posted throughout the campus of Virginia Polytechnic Institute and State University and presented in general biology and foods and nutrition classes. The flyer (Appendix B) contained a request for participants in a study of "Weight Loss Practices and Women's Health". Criteria for participation included being between the ages of 19 and 25 years, current use of weight loss practices or concern about body habitus or weight loss issues, no use of oral contraceptives or other hormone-based medication, and no current pregnancy.

Interested parties were asked to contact the primary researcher by telephone. At that time, an unstructured interview was conducted to confirm the before mentioned criteria and to inform prospective participants of their responsibilities once accepted as subjects in the study. Of 20 respondents who telephoned the primary investigator, 17 were interviewed in person in order to determine the presence of an active, diagnosable eating disorder and to review the Informed Consent form (Appendix C) which

outlined, in detail, the components of this investigation including the participants' responsibilities and compensation. The investigator reviewed information pertaining to diagnosable eating disorders in the college population and provided each potential subject with a list of student-oriented support service telephone numbers (Appendix D). Criteria for exclusion from the subject pool included the presence of active anorexia nervosa (n=2), absence of a desire for weight loss (n=1), use of a hormone-based medication within the last 6 months (n=1), and fear of needles (n=1). After the screening, 15 subjects had given their informed consent. One subject (Subject 15) was unable to continue participation due to a diagnosis of an ovarian cyst and subsequent treatment with hormone medication.

The use of human subjects for this investigation met the ethical guidelines of and was approved by the Institutional Review Board of Virginia Polytechnic Institute and State University. All participants read and signed the Informed Consent form (Appendix D) prior to participation.

### Experimental Design

The experimental design for this 90-day investigation is presented in Figure 5. Between February 7th and March 3rd, subjects were interviewed and began completion of the first set of six-day food intake and activity

records. Two subsequent six-day food intake and activity records were completed on March 13th through March 18th and on April 4th through April 9th. Subjects also documented the course of their menstrual cycles during the months of February, March, and April. Blood collection took place twice per week for five weeks starting on March 28th. Anthropometric measurements were obtained one week prior to and one week following the five week sampling period.

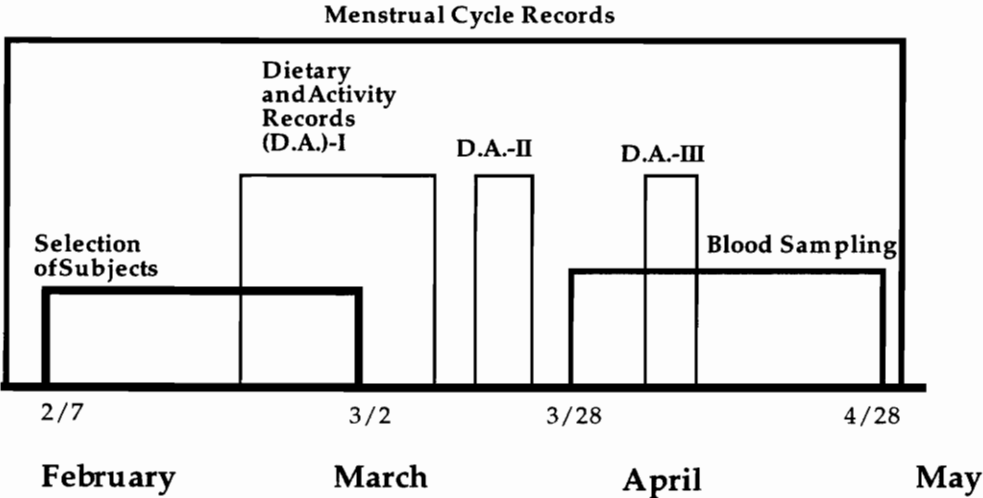


Figure 5. Experimental design.

### Initial Interview

All subjects took the self-administered EAT-26 (Appendix E) as part of the initial interview. Interview questions (Appendix F) were derived from a questionnaire used by Lydia Marek (1995) in her dissertation research and were based upon the Eating Disorder Inventory Symptom Checklist (EDI-SC) (Garner 1991). The EDI-SC has been used to assess for the presence of DSM-criteria diagnosable eating disorders. Subjects were asked to answer each question honestly and accurately and were assured confidentiality in their responses. No subjects met the criteria for diagnosis of an eating disorder. Demographic information, as well as a history of menstrual abnormalities and weight loss practices, were also collected. Subjects were assigned random identification numbers to maintain confidentiality.

### Food Intake Assessment

An average daily energy consumption for each subject was assessed using food intake records. Subjects were given an example of a completed 24-hour food intake record (Appendix G) and a photocopied schematic of approximate serving sizes (Appendix H) to increase the accuracy of recording. Subjects completed 18, 24-hour food intake records. The records were given

in three packets of six over the course of three months. Each subject's records included information from weekdays, weekend, vacation time, and different menstrual phases. The completion of food intake records in this manner decreased the likelihood that subjects would modify their diets or fabricate entries and provided a good representation of average energy intake for the individual (Terin et al. 1993). The food intake records included columns for the time of day, the description of the food or beverage, the size of the food or beverage item, and the quantity consumed. Subjects were also encouraged to make comments in a space provided at the bottom of each intake record for clarification of what was consumed or for written expression of feelings associated with the type and quantity of food or beverage consumed.

The Nutritionist III™, Version 7.2, software for MacIntosh (N-Squared Computing, Salem, OR) was used for diet record analysis. Some substitutions were made when food items could not be found in the database (Appendix I). An average daily intake of kcals was determined for each subject.

### Food-Related Behaviors and Exercise Assessment

Subjects were asked in the preliminary interview to describe the weight loss methods they most frequently used. Using this information, and incorporating behaviors and attitudes associated with diagnosable eating

disorders (such as the use of purgatives and the use of exercise to compensate for excessive energy intake), a checklist of dieting activities (Appendix J) was developed. Subjects completed this daily checklist concurrently with the dietary records previously described. Subjects were asked to provide details, where indicated, such as the type and duration of exercise used or the number of meals skipped during the day. Information collected from completed activity records was analyzed in terms of frequency of activity per one week period.

### Menstrual Cycle Assessment

Beginning February 1, subjects kept track of their menstrual flow using a calendar (Appendix K). Subjects were instructed to record , using letter codes, the presence or absence of menstrual flow, the heaviness or lightness of flow, spotting between or during periods, and length of period. These data were used to determine menstrual cycle length, luteal phase length, approximate time of ovulation, and to compare self-reported menstrual abnormalities with those determined by hormone analysis.

## Anthropometric Data

Baseline and endpoint anthropometric measurements included weight and skin-fold thickness. To minimize error, all anthropometric measurements were performed by the same lab assistant. Height without shoes was measured at baseline only. Weight was measured with subjects clothed in tee shirts and shorts and without shoes, using the same scale each time. Skin-fold measures were taken in triplicate from the suprailium, thigh, and triceps. The skin-fold measures were summed for each subject and a percent body fat was determined using the following equation:

$$\text{Body Density} = 1.0994921 - 0.0009929 (X_1) + 0.0000023 (X_1)^2 - 0.0001392 (X_2)$$

Where  $X_1$  = sum of triceps, thigh, and suprailium skin-folds and  
 $X_2$  = age in years (Jackson et al. 1980)

Midway through the 5-week blood sampling period, height and weight was measured to determine the Body Mass Index (BMI) of subjects. Desirable Body Weight (DBW) was determined using an equation from the Mayo Clinic Diet Manual (Nelson et al. 1994). The equation:

DBW = 100 lbs  $\pm$  5 lbs for every inch over 60 inches

allows for a factor of  $\pm$  10% for large or small framed females. Frame size was determined by wrist circumference and the following equation:

$$r = \frac{\text{Height in cm}}{\text{Wrist Circumference in cm}}$$

where  $r > 10.9$  for small frames,  $r = 10.9 - 9.9$  for medium frames, and  $r < 9.9$  for large frames (Zeman 1991).

### Sample Collection and Processing

Twice per week, for five consecutive weeks, approximately 7 ml of whole blood was drawn via venipuncture from the forearm of each subject. All phlebotomy procedures were performed by Janet Rinehart, MLT, ASCP. Blood was collected between 7 a.m. and 10 a.m. on Tuesdays and Fridays, beginning Tuesday, March 28th. Exceptions to this time schedule included two days when conflicts required afternoon sampling (Tuesdays 4/4 and 4/25). On four separate occasions, blood samples were taken 16 to 24 hours early



(subject 2, 4/6; Subject 9, 4/13; Subject 4, 4/20; Subject 13, 4/27). The subjects were not required to fast prior to blood sampling.

Immediately following blood collection, samples were allowed to clot for 30 minutes. Blood was centrifuged for 15 minutes at 28,000 RPM to separate serum from cells and clotting factors. Serum was extracted using Pasteur pipettes and placed in capped, labeled microtubes. Serum samples were frozen and stored at -20° C. The investigator as well as the laboratory assistant were instructed in, and did practice, safe and appropriate laboratory procedures in accordance with the Blood Borne Pathogen Program of Virginia Polytechnic Institute and State University.

#### Hormone Assays: Competitive Enzyme Immunoassay

Circulating progesterone and estradiol concentrations for each subject were determined using the Enzyme Linked Immunosorbant Assay (ELISA) method. Immunoassay kits were obtained from Cayman Chemical Company (Ann Arbor, Michigan). The assay was based upon competition between free progesterone or estradiol and an acetylcholinesterase molecule linked to progesterone or estradiol (a tracer) for a limited number of hormone-specific rabbit antiserum binding sites. The rabbit antiserum-hormone complex, containing either free hormone or tracer, bonded to a mouse monoclonal

anti-rabbit antibody which coated the wells of each microplate. Using the EL 404 Microplate Washer (Bio-Tek Instruments, Winooski, Vermont) the unbound reagents were removed, and Ellman's Reagent, which provides a substrate for acetylcholinesterase was added to the well. The product of this enzymatic reaction was measured spectrophotometrically using the Ceres 900 HDi Scanning Autoreader (Bio-Tek Instruments, Winooski, Vermont). The amount of hormone tracer that was bound to the rabbit antiserum was therefore inversely proportional to the concentration of free progesterone or estradiol in the well. The Bio-Tek Instruments program KC Jr. EIA Application Software was used to convert spectrophotometric data into concentrations of free progesterone and estradiol.

In the reconstitution of the ELISA buffer, phosphate wash buffer, hormone standard, hormone acetylcholinesterase tracer, and hormone antiserum, purified, deionized water was used. The pH of both buffers was measured after reconstitution and was found to be 7.4 for the ELISA buffer and 7.2 for the wash buffer. Standard concentrations ranged from 1000 pg/ml to 7.8125 pg/ml for estradiol and 1000 ng/ml to 7.8125 pg/ml for progesterone.

Estradiol was measured at 1/10 dilution of serum, however, a 1/5 or a 1/3.3 dilution was necessary for a few samples with lower concentrations of estradiol. For the estradiol ELISA's, the interassay variability was 9.4% and intraassay variability was 3.9%. Progesterone was measured at a 1/150

dilution, however a 1/200 dilution was necessary for samples with higher concentrations of progesterone. The interassay variability was 3.2% and intraassay variability was 4.8% for the progesterone ELISA's.

### Statistical Analysis

For each subject, maximum, minimum and mean estradiol and progesterone levels were determined. The mean estradiol and progesterone values represented the total area under the estradiol and progesterone curves. For statistical analysis, maximum, minimum and mean values were compared between subjects grouped by the following criteria:

1. Scoring above and below the designated cut-off score of 20 on the Eating Attitudes Test (EAT-26). Subjects scoring 20 or above on the EAT-26 were considered symptomatic of sub-clinical eating disorders.
2. An average daily intake of 1200 kcals or less and 1201 kcals or more, with 1200 kcals falling one standard deviation below the mean for the entire subject group.

3. Hours of moderate intensity aerobic exercise per week. There were three exercise groups: one to three hours, four to six hours, and seven or more hours per week.

Descriptive statistics were used to assess demographic data and behavioral information obtained from the initial interview. The Student's t-test was used to assess differences in pre- and post-test weights, BMI, percent body fat and differences in energy intake (Microsoft Excel Version 4.0, Microsoft Corporation, Redmond, WA).

Hormone data were entered into a statistical computer package (SAS/STAT Version 6, SAS Institute, Cary, NC) and the general linear models procedure of one way analysis of variance (ANOVA) was used to evaluate differences in maximum, minimum, and mean estradiol and progesterone values between groups. The statistical analysis package Minitab (Minitab Release 8, Minitab Inc., State College, PA) was used to perform Z-tests of two proportions on menstrual cycle dysfunction data, EAT-26 scores, and daily kilocalorie intake for the entire subject group. Menstrual cycle dysfunction was defined by anovulation, luteal phase deficiency, amenorrhea, oligomenorrhea and irregular cycles. Irregularity, amenorrhea, and oligomenorrhea were determined by review of subjects' menstrual cycle records. Anovulation and luteal phase deficiency were determined by actual

progesterone levels and menstrual cycle records combined. Length of luteal phase was defined according to parameters described by Schweiger et al. (1989) and Pirke et al. (1989). The luteal phase included all days from two days after the midcycle to the day before the start of the next menstrual period. Midcycle was defined as the day on which the follicular phase estradiol level was at its highest. For the Z-test of two proportions, categories of menstrual cycle dysfunction were numerically coded by either a yes = 1 or a no = 0. The Student's t-test (Microsoft Excel Version 4.0, Microsoft Corporation, Redmond, WA) was used to analyze differences in serum progesterone and estradiol between groups.

Correlation and regression analysis was used to evaluate the relationship between luteal phase length and energy intake. Finally, within the group of subjects scoring 20 or higher on the EAT-26, a Student's t-test (Microsoft Excel Version 4.0, Microsoft Corporation, Redmond, WA) was used to determine differences in progesterone maximum between subjects exercising seven hours per week or more and subjects exercising less than seven hours per week. An alpha level of 0.05 was considered significant in all analyses.

## CHAPTER IV

### RESULTS

Fourteen of 15 subjects selected for participation completed this investigation. Subject 15 dropped out due to diagnosis of an ovarian cyst. Descriptive characteristics are presented in Table 1.

Table 1. Descriptive characteristics of subjects.

Subject	Age	Academic Year <sup>a</sup>	Past ED Diagnosis <sup>b</sup>	EAT-26 Score <sup>c</sup>
1	22	4	N	18
2	24	5	Y	33
3	21	4	N	7
4	19	1	N	21
5	19	2	N	13
6	20	3	N	5
7	22	4	N	17
8	19	1	N	5
9	19	2	N	29
10	21	3	N	18
11	19	2	N	8
12	23	4	N	23
13	21	4	N	11
14	20	2	Y	38

<sup>a</sup> 1 = Freshman, 2 = Sophomore, 3 = Junior, 4 = Senior, 5 = Graduate.

<sup>b</sup> Y = Yes N = No

<sup>c</sup> Scores 20 points or higher are indicative of sub-clinical eating disorders.

The mean age of the subjects was  $20.6 \pm 1.6$  years. All of the subjects had currently expressed a desire to lose weight and were employing various self-selected methods to achieve this weight loss prior to participation in this study. These methods are listed in Table 2.

Table 2. Selected methods for weight loss.

Method	Number of Subjects
Skipping Meals/Fasting	4
Restricting/Counting Fats	13
Restricting/Counting Kilocalories	12
Restricting Sweets	9
Exercise	5
Fad Diets	1

Although all subjects except one (Subject 6) had a BMI below  $25 \text{ kg/m}^2$ , which according to Nelson et al. (1994) is equivocal to normal weight, 71% of subjects reported that at their present weight they felt they were “somewhat overweight” and 86% indicated that they would feel “somewhat negative” about a two pound gain in body weight. All subjects reported that they would feel “somewhat” to “extremely positive” about a two pound loss of body weight.

Thirty-six percent of subjects scored above the symptomatic cut-off of 20 points on the EAT-26. This group of five individuals included two subjects with previous clinical diagnoses of anorexia nervosa (Subject 14) and bulimia nervosa (Subject 2). These two subjects had undergone treatment for their eating disorders and at the time of this investigation, no participants met the DSM-IV criteria for anorexia or bulimia nervosa. Based on scores on the EAT-26, two groups, the Sub-Clinical (SC) group (n=5) and the Normal Dieter (ND) group (n=9) were formed. The mean number of years that subjects had been dieting for weight loss prior to participation in this study was  $4.6 \pm 3.3$  years for the ND group and  $5.8 \pm 5.6$  years in the SC group. The difference was not statistically significant.



Anthropometric Measures

The mean baseline and post-test weights, percent of desirable weight (DBW), and percent body fat for the SC and ND groups are listed in Table 3.

Table 3. Mean baseline and post-test weights, percent of desirable weight, and percent body fat for subjects. (Mean  $\pm$  SD)

Anthropometric Measures	SC (n=5)	ND (n=9)
Baseline Body Weight (lbs)	129.3 $\pm$ 9.4 <sup>a</sup>	146.7 $\pm$ 11.6 <sup>b</sup>
Post-Test Body Weight (lbs)	128 $\pm$ 8.8 <sup>a</sup>	146 $\pm$ 11.5 <sup>b</sup>
Desirable Body Weight (lbs)	122.5 $\pm$ 6.0 <sup>c</sup>	136 $\pm$ 11.6 <sup>d</sup>
Baseline % of Desirable Body Weight	105.7	107.9
Post-Test % of Desirable Body Weight	105	107.8
Baseline % Body Fat	22.5 $\pm$ 2.2	26 $\pm$ 1.4
Post-Test % Body Fat	22.1 $\pm$ 1.9	25.3 $\pm$ 1.3

<sup>a,b</sup> (p < 0.02)

<sup>c,d</sup> (p < 0.03)

While there was a significant difference between groups for body weight at baseline (p < 0.02) and for post-test measures (p < 0.02), the difference was not statistically significant when weights were analyzed as a percentage of calculated desirable body weight. This was due to the fact that the ND group had a significantly higher calculated desirable weight based on height and

frame size than the SC group ( $p < 0.03$ ). Although the percent body fat measures were greater in the ND than in the SC subjects at both baseline and at post-test measurements, this difference was not statistically significant. Most importantly, there was no significant change in body weight or percent body fat within subject groups during the course of this investigation.

The Body Mass Index determined midway through the five week blood sampling period ranged from 19.86 kg/m<sup>2</sup> to 26.93 kg/m<sup>2</sup>. Mean BMI in the ND group ( $22.6 \pm 2.0$ ) was slightly higher than the mean BMI for the SC group ( $21.1 \pm 1.5$ ), however the difference was not significant.

### Menstrual History

Subjects in the ND group reported a history of a greater number of menstrual cycle abnormalities since menarche than was reported by the SC subjects. Table 4 illustrates the percentage of subjects who had a self-reported history of amenorrhea, oligomenorrhea, diminished menstrual blood flow, and diet-related menstrual changes.

Table 4. History of menstrual cycle abnormalities in SC and ND subjects.

	SC	ND
Menstrual Cycle Abnormalities	(n=5)	(n=9)
Amenorrhea	0	44%
Oligomenorrhea	20%	44%
Diminished Menstrual Blood Flow	40%	67%
Diet-Related Menstrual Changes	40%	67%

Subjects who reported a history of diet-related menstrual changes named reduced dietary intake, weight loss, irregular eating patterns due to a busy schedule, emotional stress, or increased intake of complex carbohydrates as the cause of their disrupted cycles. During the course of this investigation, two subjects were amenorrheic (Subject 5 and Subject 8), no subjects were oligomenorrheic, and two subjects experienced irregular cycles (Subject 9 and Subject 11). Of these subjects, only Subject 9 represented the sub-clinical group. All of these subjects had reported a history of oligomenorrhea.

## Food Intake Records

Subjects consumed a mean intake of  $1687 \pm 571$  kcals per day. The SC subjects tended to consume less kcals ( $1408 \pm 170.4$ ) than the ND ( $1841 \pm 205.7$ ), however the difference was not statistically significant ( $p = 0.13$ ). All subjects except one (Subject 8) had an average daily energy intake, as measured as kcals consumed per kilogram of body weight, which was less than 38 kcal/kg of body weight, the published Recommended Energy Allowance for this age group (National Research Council 1989). As a fraction of their body weight in kilograms, the SC and ND groups had similar caloric intakes ( $21.1 \pm 2.4$  and  $22.6 \pm 4.0$  kcal/kg body weight, respectively). During the course of this investigation, many subjects from each group frequently consumed less than 1200 kcals per day. Table 4 lists the mean daily caloric intake for each subject as well as the low and high values.

Table 5. Low, high, and mean daily energy intake among subjects.

Number	High	Low	Mean $\pm$ SD
1	4437	523	2631.7 $\pm$ 1137
2*	2601	1355	1922 $\pm$ 396.9
3	2222	750	1484 $\pm$ 417.8
4*	2103	647	1298 $\pm$ 435.3
5	2358	1045	1790 $\pm$ 376.1
6	2683	856	1571.2 $\pm$ 536.7
7	2673	1314	1847 $\pm$ 412.9
8	4388	2044	3051 $\pm$ 714.8
9*	1874	257	1154 $\pm$ 456.5
10	3342	777	1673 $\pm$ 664.8
11	2297	408	1432 $\pm$ 561.5
12*	2349	1009	1669.6 $\pm$ 397.5
13	2290	401.4	1093 $\pm$ 472.5
14*	1628	679	994 $\pm$ 263.7

\* SC Group

While the low values for caloric intake were lower in the SC group when compared to the ND group, this difference was not significant ( $p = 0.67$ ). The high values for the SC group were, however, significantly lower than the high values for the ND group ( $p < 0.03$ ).

## Dieting and Activity Records

Table 6 lists the weight loss practices and food-related behaviors employed most frequently by the SC and ND subjects during the course of this investigation. Activities are listed according to number of times or hours per week, and two items, exercise and meal skipping, are listed by motivation as well. The data illustrate that the SC subjects were more likely than ND subjects to skip meals to reduce caloric intake, to use weight loss supplements, and to use purging behaviors such as self-induced vomiting in the attempt to lose weight. The frequency with which the ND subjects exercised for weight loss and to maintain fitness was similar to that of the SC subjects. Normal Dieter and SC subjects skipped meals with similar frequency, however the motives appeared to differ as the SC subjects reported purposely skipping meals for weight loss and the ND subjects reported skipping meals due to busy schedules. The frequency of binge eating was greater in the SC subject group. No test of significance was used to analyze these data as small frequencies gave limited power.

Table 6. Reported frequency of dieting and food-related behaviors.

Activities	Frequency (times/hours per week)*			
	0	1-3	4-6	7+
<b>Meal Skipping for Weight Loss</b>				
SC (n = 5)	2	2	1	0
ND (n = 9)	7	2	0	0
<b>Meal Skipping Due to Busy Schedule</b>				
SC	2	2	1	0
ND	2	3	3	1
<b>Calorie Counting</b>				
SC	4	1	0	0
ND	7	2	0	0
<b>Fat Gram Counting</b>				
SC	5	0	0	0
ND	7	2	0	0
<b>Skipping Dessert</b>				
SC	3	2	0	0
ND	5	4	0	0
<b>Use of a Weight Loss Supplement</b>				
SC	4	1	0	0
ND	9	0	0	0
<b>Exercise to Compensate for Food Intake</b>				
SC	4	0	1	0
ND	5	2	1	0
<b>Exercise to Maintain Fitness</b>				
SC	0	1	2	2
ND	1	2	6	0
<b>Self-Induced Vomiting</b>				
SC	4	1	0	0
ND	0	0	0	0
<b>Use of Laxatives or Diuretics</b>				
SC	0	0	0	0
ND	0	0	0	0
<b>Binge eating</b>				
SC	2	2	1	0
ND	5	4	0	0

\* Frequency is listed as the number of times per week (or in the case of exercise, number of hours per week) per subject group.

### Serum Estradiol and Progesterone and EAT-26 Scores

Table 7 lists the minimum, maximum, and mean serum progesterone and serum estradiol levels for both the SC and ND groups. The mean values for estradiol and progesterone represented the area under the hormone curves.

Table 7. Minimum, maximum and mean serum progesterone and estradiol levels during the course of one menstrual cycle. (Mean  $\pm$  SD)

	SC (n=5)	ND (n=9)
Progesterone (ng/ml)		
Minimum	14.72 $\pm$ 6.74	21.24 $\pm$ 21.36
Maximum	64.3 $\pm$ 31.08	83.24 $\pm$ 51.68
Mean	30.33 $\pm$ 12.43	39.63 $\pm$ 27.65
Estradiol (pg/ml)		
Minimum	98.36 $\pm$ 45.73	109.11 $\pm$ 56.04
Maximum	312.58 $\pm$ 66.14	333.67 $\pm$ 107.54
Mean	191.70 $\pm$ 58.53	198.99 $\pm$ 66.83

There were no significant differences between groups on any of these variables ( $p = 0.47$ ). When group serum hormone values were compared according to the phase of the menstrual cycle, a significant difference between mean serum progesterone values during the follicular phase was found. In



the follicular phase, mean serum progesterone levels were significantly lower in the SC group ( $21.6 \pm 2.1$  ng/ml) as compared with the ND group ( $37.6 \pm 4.9$  ng/ml) ( $p < 0.004$ ). During the luteal phase, mean serum progesterone values were again lower in the SC group ( $40.5 \pm 5.2$  ng/ml) than those found in the ND group ( $55.6 \pm 5.7$  ng/ml) ( $p = 0.07$ ). In both phases of the menstrual cycle, there were no significant differences found between groups for serum estradiol values.

Figure 5 illustrates the cyclic ovarian hormone patterns for all subjects. Figure 1 on page 11 of this text illustrates a normal menstrual cycle and can be used as a reference for comparison. A total of five women (36%) experienced luteal deficiency. Subjects 2 and 9 represented the SC group and Subjects 5, 8, and 11 represented the ND Group. The subjects who were amenorrheic (Subject 5 and Subject 8) or who had irregular cycles (Subject 9 and Subject 11) during the course of this investigation also experienced luteal phase deficiency as characterized by the absence of a luteal phase progesterone peak. Subject 2, while presenting a regular, ovulatory cycle, also appeared to experience luteal deficiency with no apparent rise in serum progesterone after ovulation. The approximate time of ovulation is indicated by a downward arrow on each hormonal plot in Figure 5. For Subjects 5, 8, 9 and 11, approximate time of ovulation could not be determined due to irregular menstrual bleeding (Subject 9 and Subject 11) or lack of menstrual bleeding

(Subject 5 and Subject 8). The serum progesterone and estradiol values for these subjects were also not indicative of ovulation, however, ovarian ultrasonography is the only accurate measure of this occurrence, and it is therefore uncertain if these subjects experienced ovulatory cycles even in the absence of menstrual bleeding. Subject 2 had both serum estradiol peaks and menstrual bleeding which were indicative of ovulation.

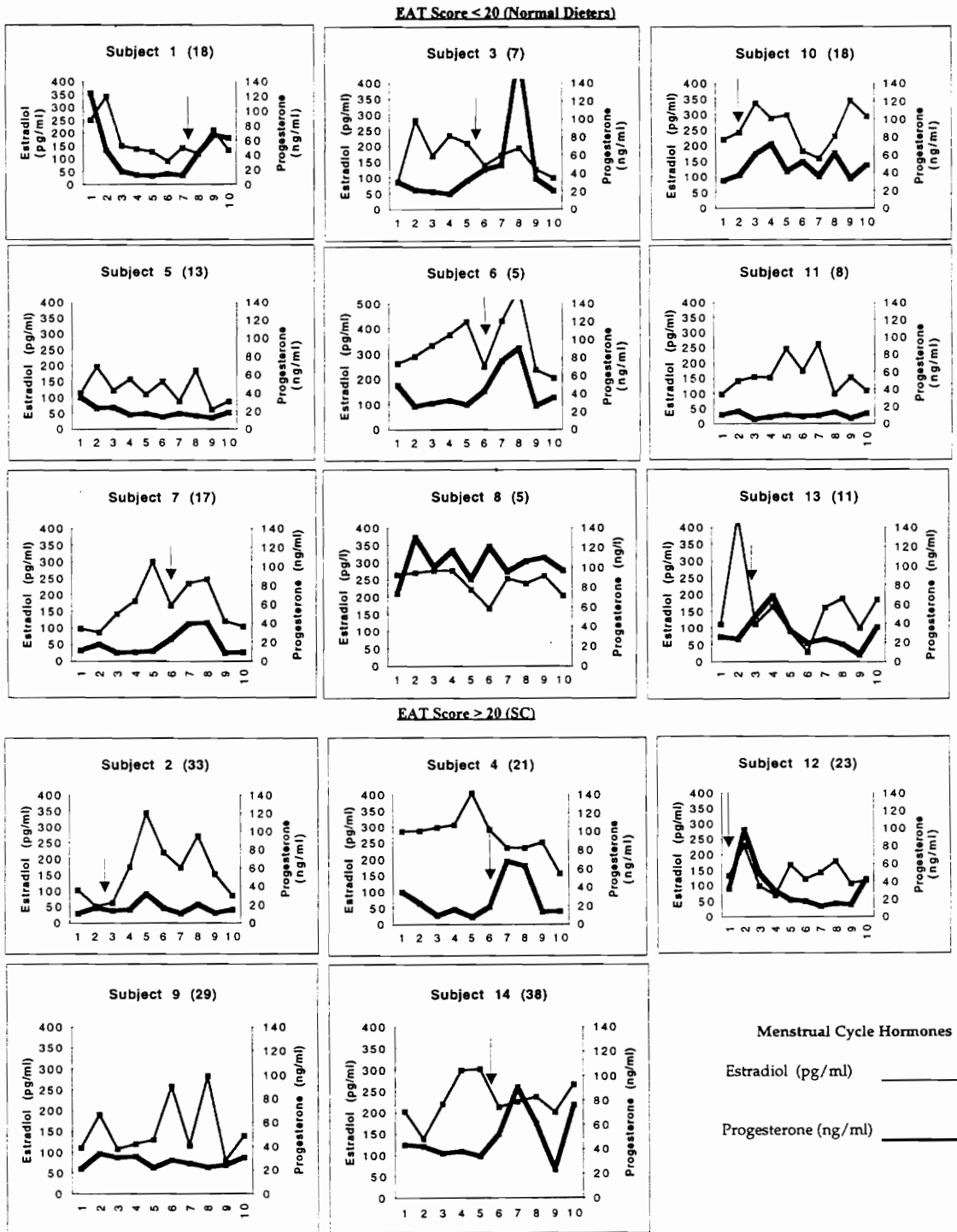


Figure 6. Serum progesterone and estradiol values across one menstrual cycle.

### Length of Luteal Phase and Daily Energy Consumption

Subjects were divided according to average daily kilocalorie consumption. Three subjects (9, 13 and 14) fell within the category of those consuming an average of 1200 kcals/d or less. There was a trend ( $p = 0.07$ ) for a shortened luteal phase length (Days  $\leq 10$ ) or apparent absence of luteal phase with decreased average daily caloric consumption (kcals  $\leq 1200$ ). Luteal phase lengths and average daily energy consumption for each subject are listed in Table 8.

Table 8. Length of luteal phase and mean energy intake per day of all subjects.

Subject	Length of Luteal Phase (Days)	Mean Energy Intake/Day (kcals)
1	12	2631
2	14	1922
3	13	1484
4	13	1298
5	*	1790
6	13	1571
7	14	1847
8	*	3051
9	7	1154
10	10	1673
11	8	1432
12	13	1670
13	10	1093
14	12	994

\* Unable to determine luteal phase length.

For Subjects 5 and 8, due to absent menses and non-definitive serum hormone values, the length of luteal phase could not be determined.

A regression analysis of the correlation between average energy intake and luteal phase length in 12 of 14 subjects indicated a non-significant positive relationship ( $r = 0.384$ ,  $r^2 = 0.147$ ,  $p = 0.21$ ) between these two variables. A scatterplot diagram of this relationship is found in Figure 6.

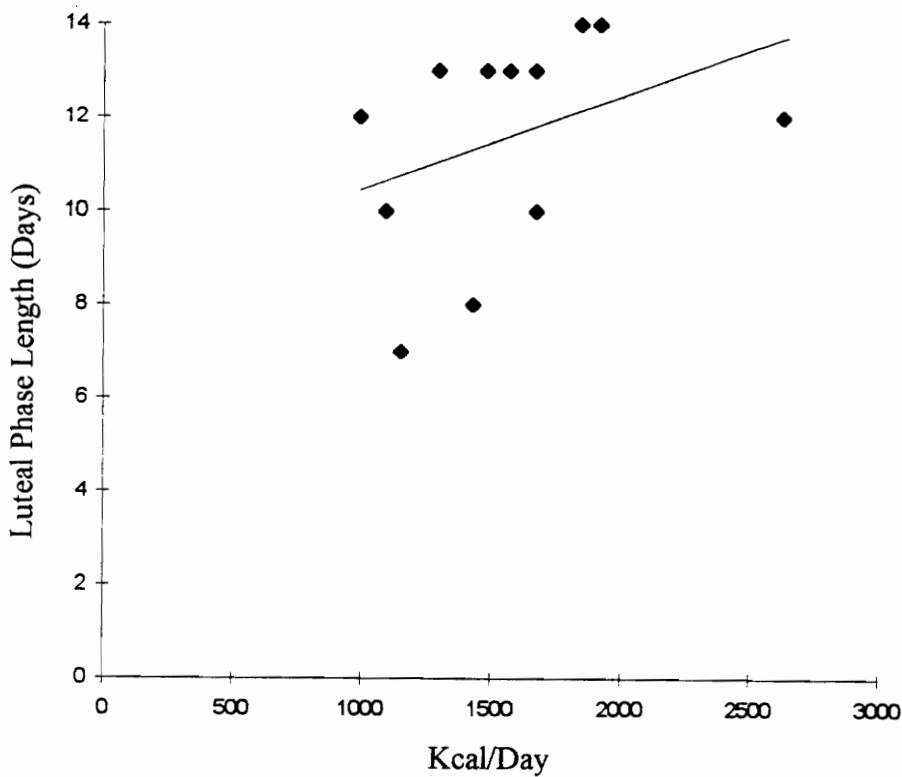


Figure 7. Relationship between luteal phase length and kcals consumed per day.

There was no significant relationship found between average daily energy intake and other indices of menstrual cycle dysfunction.

### Serum Estradiol and Progesterone and Frequency of Exercise

As noted previously, level or frequency of activity did not differ between the SC and ND groups. When all subjects were divided according to frequency of low to moderate intensity aerobic exercise, serum estradiol and progesterone values appeared to be affected. Table 9 lists the minimum, maximum and mean serum estradiol and progesterone levels in subjects divided according to frequency of exercise in a one week period. Four subjects (Subjects 1, 3, 4 and 10) participated in low to moderate intensity aerobic activity 1 - 3 hours per week (Group 1), 8 subjects (Subjects 5, 6, 7, 8, 11, 12, 13 and 14) participated 4 - 6 hours per week (Group 2), and 2 subjects (Subjects 2 and 9) participated 7 or more hours per week (Group 3).

Maximum or peak serum progesterone values were lower in subjects who exercised most frequently. The difference between Group 1 and Group 3 values was significant ( $p < 0.03$ ), the greatest level of exercise having the lowest serum progesterone maximum. There was a trend ( $p = 0.06$ ) for lowered mean serum progesterone in the more frequent exercisers (Group 3) as compared with Group 1 subjects, who exercised the least. While mean

serum estradiol levels were less (218.8 pg/ml) in Group 1 than in Group 2 (158.5 pg/ml), this difference was not significant ( $p = 0.06$ ). There were no significant differences in serum estradiol values between groups.

Among members of the SC group, there was a significant difference in the maximum serum progesterone level and in the difference between maximum and minimum serum progesterone between subjects who exercised 7 hours per week or more (Subjects 2 and 9) and subjects exercising 1 - 6 hours per week (Subjects 4, 12, 14). Figure 7 illustrates luteal phase deficiency among frequently exercising SC subjects. The maximum and minimum serum progesterone values for these subjects and for Subjects 4, 12, and 14, who exercised between 1 and 6 hours per week, are listed in Table 10. In subjects who exercised the most frequently, maximum serum progesterone levels were significantly lower ( $32.5 \pm 1.9$  ng/ml) than maximum serum progesterone levels in SC subjects who exercised less frequently ( $85.5 \pm 15.6$  ng/ml) ( $p < 0.03$ ). The difference between maximum and minimum serum progesterone values was also significantly lower in frequent exercisers ( $17 \pm 5.4$ ) than in SC subjects who exercised less frequently ( $70.8 \pm 14.1$  ng/ml) ( $p < 0.03$ ).

Table 9. Serum progesterone and estradiol levels across differing exercise frequencies.

Level of Exercise*	N	PMIN		PMAX		PMEAN		EMIN		EMAX		EMEAN	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	4	16.8	9.9	109.4	50.1 <sup>a</sup>	39.8	9.0 <sup>c</sup>	123.5	39.3	343.6	50.3	218.8	56.1
2	8	20.9	22.5	71.0	38.5	38.3	29.7	106.1	58.9	320.5	119.6	194.6	70.9
3	2	15.5	7.4	32.5	1.9 <sup>b</sup>	21.3	7.9 <sup>d</sup>	65.5	19.0	313.8	42.7	158.5	7.2

\*Level of Exercise - Low to Moderate Intensity, Aerobic (Stairmaster, Step Aerobics, Walking):

Group 1 1-3 Hours/Wk

Group 2 4-6 Hours/Wk

Group 3 7+ Hours/Wk

<sup>a,b</sup> ( $p < 0.03$ )

<sup>c,d</sup> ( $p \geq 0.06$ )

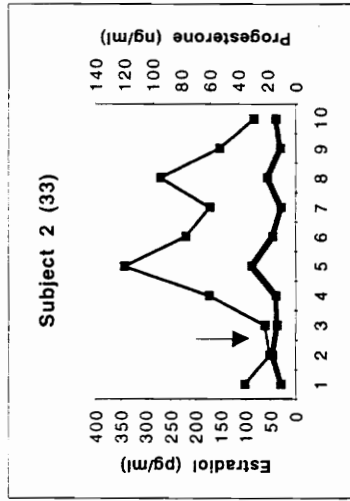


Table 10. Maximum and minimum serum progesterone levels in SC subjects.

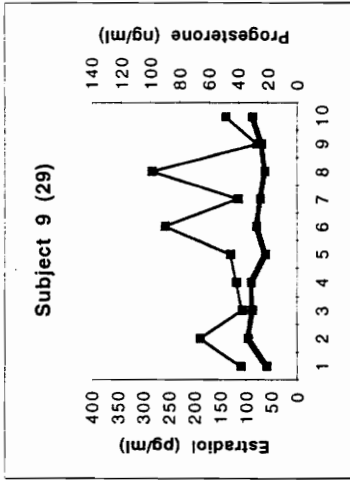
Subject	Maximum (ng/ml)	Minimum (ng/ml)	Difference (Max-Min)
2*	31.1	10.3	20.8
4	67.9	9.3	58.6
9*	33.9	20.7	13.2
12	97.8	11.6	86.2
14	90.8	23.1	67.7

\*Exercise = 7+ hours per week

7 + Hours Activity/Week

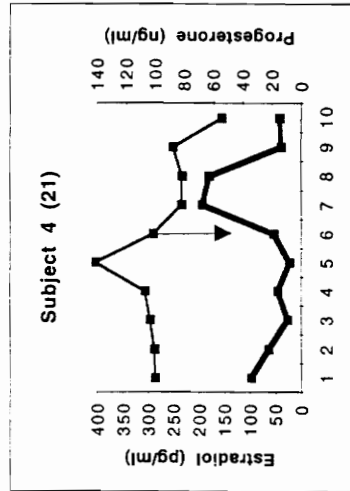


Emin=52, Emax=344 Pmin = 10.3, Pmax=31.1

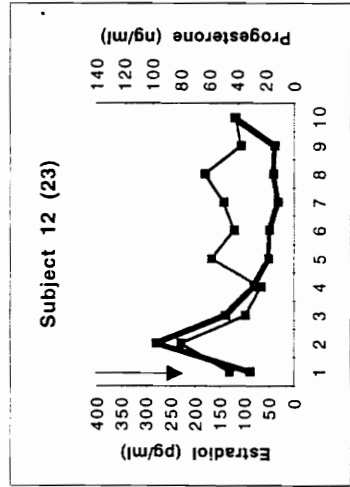


Emin=78.9, Emax=283.6 Pmin=20.7, Pmax=33.9

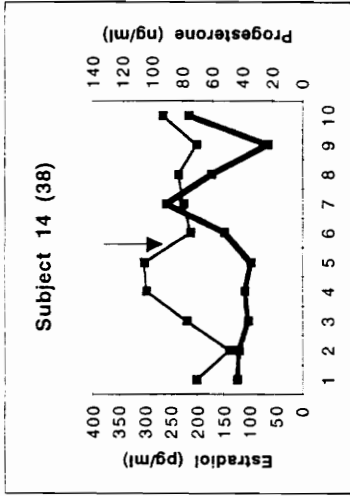
1 - 6 Hours Activity/Week



Emin=155.5, Emax=404.8 Pmin=9.3, Pmax=67.9



Emin=67.1, Emax=228.6 Pmin=11.6, Pmax = 97.8



Emin=138.3, Emax=301.9 Pmin=23.1, Pmax=90.8

Figure 8. Serum estradiol and progesterone values for SC group according to exercise frequency.

## CHAPTER V

### DISCUSSION AND CONCLUSIONS

#### Introduction

This study presents the results of an analysis of energy intake, level of activity, EAT-26 score, and serum estradiol and progesterone levels for 14 normal weight, college-age women who were dieting to lose weight. Of these 14 subjects, 36% scored above the symptomatic cut-off score of 20 points on the EAT-26 and were therefore considered to hold the attitudes and beliefs of individuals with sub-clinical eating disorders. Included among those who scored above 20 points were two subjects (2 and 14) who had been previously diagnosed with and treated for bulimia nervosa and anorexia nervosa, respectively. This rate of 14% of subjects with previous clinical diagnoses of eating disorders is considerably higher than the national average for adolescent and college-age females, which is between 0.5 and 3% according to the fourth edition of the DSM (American Psychological Association 1994). The subjects participating in this study, however, cannot be considered representative of college-age women as a whole due to the small sample size and to the requisite that for inclusion in this study, subjects had to be of normal weight for height and had to maintain a desire for weight loss.

Upon initial interview, 71% of all subjects reported that at their present weight they felt they were “somewhat overweight” and 86% indicated that they would feel “somewhat negative” about a two pound gain in weight. Given that subjects were within 10% of DBW, one might expect that attitudes, beliefs, and behaviors associated with disordered eating might be higher in this group of subjects (Whitaker 1992).

Although studies of menstrual cycle function have been conducted with infertile patients who have dieted to maintain a thin body habitus (Bates 1982) and with women who experienced simple weight loss (Pirke et al. 1989), this group who experienced no loss in body weight or body fat despite attempts to lose weight and also experienced menstrual dysfunction has previously received little attention.

### Assessment of Compliance

Beginning with the initial interview, the primary investigator attempted to establish an open and friendly rapport with each subject and maintained contact with subjects through written correspondence and biweekly phone conversations. Subjects were encouraged to visit or call the primary investigator with concerns about the investigation. Compliance of subjects was assessed by way of anonymous responses to a written survey

(Appendix L). All subjects reported that they completed food intake records, activity and dieting behavior records, and menstrual cycle records honestly and accurately throughout the course of this study. Original protocol of this investigation included the keeping of basal body temperature for the assessment of time of ovulation. Five subjects reported that they had falsified data on days when they forgot to take basal temperatures. The use of temperature data was, therefore, excluded from the analysis of the results. Because subjects were willing to admit to this deviation from the study design, this researcher has reason to believe that the falsification of other records would have been reported had it occurred. The rate of attrition in this investigation was 0%.

### Factors Affecting the Menstrual Cycle

Of the SC and ND subjects, it was the ND subjects who had experienced a greater number of previous menstrual cycle abnormalities. Forty percent of SC subjects and 67% of ND subjects claimed they had experienced diet-related menstrual changes. The true etiology of these changes might have resulted from underlying abnormalities unrelated to dieting behavior. If they were strictly diet-related, the greater occurrence of menstrual cycle changes in the ND group may have indicated that certain individuals were more

physiologically sensitive to dietary intake and activity. The serum estradiol and progesterone levels in ND subjects who may have had an underlying endocrine dysfunction could have confounded the results. However, within the scope of this investigation, it was not feasible to screen subjects for abnormal endocrine function through laboratory assay. Despite the high prevalence of menstrual abnormalities in both the ND and the SC subjects, no subject had been clinically diagnosed with menstrual cycle dysfunction of primary origin according to the initial interview. Exclusion of subjects with a history of menstrual cycle dysfunction might have limited the number of subjects further due to the fact that even simple weight loss within a range normal for height has been shown to induce menstrual hormone changes in women having no apparent eating pathology (Pirke et al. 1986, Schweiger et al. 1987, Pirke et al. 1989).

Of the 14 female subjects, 5 (36%) experienced dysfunctions of the menstrual cycle including amenorrhea, irregular menses, luteal phase deficiency, and possible anovulation (Speroff et al. 1978) during this investigation. This rate of dysfunction could not be solely attributed to a loss of body weight or body fat in these subjects. Only one subject experiencing menstrual cycle dysfunction had a body fat percent below that established by Frisch and MacArthur (1974) to be necessary for the maintenance of menstrual periods (22%). Subject 9 was measured at 17% body fat at both

baseline and post-test. Subject 9 did not have a history of amenorrhea but had experienced oligomenorrhea prior to inclusion into this study. During the course of this investigation her menstrual periods were irregular; This may have resulted from a body fat percentage inadequate to support menstrual cyclicity. Serum estradiol level has been previously shown to be directly related to body fat percentage (Frisch and MacAurthur 1974). There is a close dependence on body fat stores in the conversion of the androgens testosterone and androstenedione to estrogen. However, progesterone metabolism does not seem to be closely associated with percent body fat. Progesterone is synthesized by the lutenized granulosa cells of the corpus luteum during the luteal phase (Terin et al. 1993). It is interesting to note that despite a low body fat, Subject 9 was able to maintain serum estradiol levels within the published normal range of 23 to 361 pg/ml (Tilkian et al. 1987) and achieved peak serum estradiol levels (Figure 5) well above the normal minimum of 120 pg/ml used by Schweiger et al. (1987) in their investigation of diet-induced menstrual irregularities and by Pirke et al. (1987) in their study of normal weight bulimics.

No subject experiencing menstrual cycle dysfunction weighed less than 104.8% or more than 110.2% of DBW at baseline and post-test measurements. In fact, Subject 9 weighed 104.3% of DBW at baseline and 104.9% of DBW at post-test and therefore, would not have been considered underweight, but

may have been functionally “under-fat” (Frisch and MacAurthur 1974). This suggests the possibility that other subjects experiencing menstrual cycle dysfunction may have also been functionally underweight or under-fat, despite being within a normal range of a calculable DBW and above 22% body fat. This hypothesis has been previously proposed (Kreipe et al. 1989) and suggests that reproductive health may be affected in women whose body weight falls below their physiological “normal” weight. When 37 fertility clinic patients, who had been dieting for weight loss, were encouraged to gain weight to a predicted IBW, 76% were able to conceive and 90% with secondary amenorrhea resumed regular menstruation (Bates et al. 1982).

Mid-study BMI measures confirmed that while all subjects reported a desire for weight loss, 13 out of 14 of the subjects had BMI’s between 19.8 and 23.6 kg/m<sup>2</sup>, a range considered normal (Nelson et al. 1994). Subject 10, who experienced a normal menstrual cycle, had a BMI of 26.9 kg/m<sup>2</sup>, which is considered overweight, but not obese (Nelson et al. 1994). These anthropometric results support the findings of Levy, et al. (1989), Cantopher, et al. (1988), and Pirke, et al. (1987) who found that as many as 50% of normal weight bulimics experienced patterns of menstrual cycle dysfunction similar to those of the underweight anorectic.

Serum estradiol levels for all subjects were within or exceeded the published normal range. There were no significant differences in serum



estradiol levels between SC and ND subjects nor between subjects who did and did not experience menstrual dysfunction. Therefore, despite diminished progesterone values and absence of a characteristic luteal phase progesterone peak, biphasic estradiol levels in most subjects were indicative of ovulation. As noted previously, the interassay and intraassay variability for the estradiol ELISA was 9.4% and 3.9%, respectively, therefore values attained were reliably accurate. The interassay and intraassay variability for the progesterone assay were 3.2% and 4.8%, respectively, however, unlike the values obtained for estradiol, progesterone values were not consistent with the published normal ranges of 0.2 to 0.6 ng/ml during the follicular phase and 6.5 to 32.2 ng/ml during the luteal phase (Tilkian et al. 1987). As noted in Figure 5, the progesterone values, while demonstrating the characteristic luteal phase peak in normally menstruating subjects, were consistently elevated across the menstrual cycle as compared to normal published values. Although giving seemingly elevated results, each sample was assayed two times in duplicate for progesterone concentration. Because analysis of results in this investigation involved a comparison of peak, minimum, and mean serum estradiol and progesterone values instead of a comparison of the values to a normal range, deviation from published normal values was not considered a limiting factor.

Whole blood samples were taken twice per week for five weeks. Therefore, the absence or presence of a biphasic estradiol curve and monophasic progesterone curve might have reflected infrequent blood sampling as opposed to actual ovarian cyclicity. For example, Subject 5 (Figure 5) appears to have not experienced a rise in progesterone nor the characteristic biphasic estradiol curve as compared with the diagram depicting hormone values for Subject 6. The question arises that if blood sampling had occurred more frequently, would the hormonal curves have been altered? Previous investigators (Schweiger et al. 1987, Pirke et al. 1987, Cantopher et al. 1988) with similar research hypotheses and experimental designs conducted blood sampling for hormone assay anywhere from once every 2 weeks to once per day over the course of two menstrual cycles. Results were similar between these investigations. It was assumed, therefore, that the frequency of sampling in this investigation was adequate.

In addition to its role in the preparation of the endometrium for implantation of the fertilized ovum and maintenance of pregnancy, progesterone has several important functions. According to Prior et al. (1990) progesterone is active in bone metabolism through promotion of bone formation and acceleration of remodeling. In a study of 66 premenopausal women, Prior et al. (1990) found an association between inadequate production of progesterone and accelerated bone loss, despite normal

production of estradiol. This same hormonal pattern was seen in 36% of subjects from the present study however, it could not be determined through this investigation if bone density was affected. Progesterone also induces an elevation in basal body temperature (Jones and Jones 1981). This thermogenic effect is thought to be centrally mediated and has not been previously measured in the sub-clinical or normal dieting population. Finally, through the formation of hormone-receptor complexes which binding to specific DNA sequences on the chromatin, thus controlling mRNA synthesis, progesterone regulates the production of certain proteins including uteroglobin and the enzyme aldolase (Catt et al. 1987). Deficient levels of progesterone may have an impact which goes beyond menstrual dysfunction or overt infertility.

In contrast to the findings of Rippon et al. (1988), the results of this investigation indicated that the EAT-26, alone, did not serve as a sensitive indicator of menstrual hormone status. Of the 5 subjects experiencing dysfunctions of the menstrual cycle, only two had scored above the symptomatic cut-off of 20 points on the EAT-26 (Subject 2 and Subject 9). The remaining 3 subjects (Subjects 5, 8 and 11) scored 13, 5, and 8 points, respectively. Because subjects with menstrual cycle dysfunction represented both SC and ND groups, differences between SC and ND subjects were not significant. This contrasts previous investigations of the prevalence of menstrual disturbances among women with sub-clinical eating disorders

(Kreipe et al. 1989). The results of Kreipe et al. indicated that as many as 66% of normal weight females scoring above 20 on the EAT-26 (n=15) had a history of amenorrhea, whereas none of the control subjects reported a history of amenorrhea. In this same study, both sub-clinical subjects (27%) and controls (11.6%) had experienced oligomenorrhea. Unlike the preliminary investigations (Rippon et al. 1988, Kreipe et al. 1989) of the occurrence of menstrual cycle dysfunction among the sub-clinical populations, which relied on self report of menstrual history, the present investigation relied on current menstrual records and actual serum estradiol and progesterone values for analysis. Therefore, it is not surprising that results differ between this and previous studies.

Mean serum estradiol and progesterone values between SC and ND groups were not significantly different across one menstrual cycle, however, when group mean serum progesterone values were compared according to phase of the menstrual cycle, the SC group had lower mean serum progesterone than the ND group in both the follicular and luteal phases. These results are similar to those of Schweiger et al. (1992) who found that among restrained eaters there was a significantly lower luteal phase serum progesterone than in unrestrained eaters. The present study provides no data to confirm or refute high cognitive restraint among the SC subjects, however,

dietary energy intake was somewhat lower among SC subjects than ND subjects ( $1408 \pm 170.4$  kcals vs  $1841 \pm 205.7$  kcals,  $p = 0.13$ ).

Among four subjects experiencing disturbances of cyclic ovarian function (2, 5, 9, and 11) luteal phase deficiency was the common denominator. The absence of a distinct serum progesterone peak during the luteal phase in these subjects indicates several possible problems. If luteal phase serum progesterone can be used to indicate ovulation (Speroff et al. 1978), these subjects may have failed to ovulate. Secondly, they may have experienced diminished effectiveness of the corpus luteum to secrete progesterone possibly due to inadequate LH receptor sites. Finally, they may have had insufficient pituitary secretion of LH which serves to stimulate the secretion of progesterone from the corpus luteum. Results similar to these were found in previous investigations of normal weight bulimic women (Pirke et al. 1987), untrained women who exercise strenuously (Bullen et al. 1985), and restrained eaters (Schweiger et al. 1992). The etiology of these menstrual abnormalities in normal weight dieters and those with sub-clinical eating disorders remains uncertain.

Recent research has suggested that menstrual cycle dysfunction in normal weight women with eating disorders may be promoted by an underlying metabolic response to starvation during periods of purposeful caloric restriction (Mitchell, et al. 1983, Cantopher et al. 1988, Kaye et al. 1990,

and Schweiger et al. 1992). Because bulimic individuals, restrained eaters, and chronic dieters may use fasting or very low kilocalorie diets as a means to achieve weight loss, it is possible that the body interprets sub-optimal energy intake in the same manner that it interprets starvation. Likewise, when previously untrained women begin strenuous exercise programs without correcting dietary intake for increased energy needs, the body may respond through metabolic adaptations which have repercussions on ovarian hormone status. Norepinephrine and serotonin, neurotransmitters which play a role in the regulation of GnRH secretion from the hypothalamus, are especially sensitive to deficient energy intake (Schweiger 1991). Insufficient LH secretion from the pituitary would result from failure of the hypothalamus to secrete GnRH. One explanation for this may be the central effect of sub-optimal energy intake but this requires extensive future research.

There was a trend in this study toward a shortened length of luteal phase in subjects who consumed the lowest average number of kilocalories per day. While dietary restraint was not directly measured, only 3 out of 14 subjects consistently consumed more than 1200 kcals on each day of the study. Eight out of 14 subjects consumed less than 1000 kcals per day at least once during the course of this investigation. When subjects were initially interviewed and asked about current weight loss methods, 4 out of 14 said that they regularly skipped meals in order to lose weight, and 13 out of 14

said that they restricted fat and kilocalories. The extent of this restriction and the possible effect it may have had on the hormone function cannot be determined through the results presented here. However, shortened luteal phase length is considered a subcategory of luteal phase deficiency, and therefore insufficient energy intake cannot be ruled out as a possible contributing factor to the menstrual cycle dysfunction seen in this investigation.

As a ratio of kcals per kilogram of body weight, the subjects who experienced dysfunction of the menstrual cycle were not consuming more or less than those who had normal cycles. However, it should be noted that only Subject 8 consumed more than the recommended 38 kcal/kg of body weight (National Research Council 1989), with the mean being  $26.2 \pm 21$  kcal/kg for all subjects. If, as a group, subjects were consuming sub-optimal energy intakes, thereby eliciting a metabolic response such as that previously described (Schweiger 1991), then this may explain the even distribution of menstrual cycle abnormalities across SC and ND groups. Of the two SC subjects who experienced menstrual cycle abnormalities, only Subject 9 had an average energy consumption less than 1200 kcals. The four other subjects experiencing menstrual cycle dysfunction consumed an average number of kcals greater than 1200.

The frequencies of self-induced vomiting and the use of pharmacological purgatives were limited among both SC and ND subjects. Only one subject (14) induced vomiting once during the study and no subjects reported using laxatives or diuretics. The hypothesis that these behaviors would be associated with menstrual cycle dysfunction could therefore not be validated. There were, however, 5 subjects who reported using exercise to compensate for food intake (1, 7, 8, 9, and 13). Of these, Subjects 8 and 9 experienced menstrual cycle dysfunction, specifically, luteal phase deficiency.

The results of the analysis of exercise frequency and menstrual cycle function indicated that there was an association between frequency of low to moderate intensity aerobic activity and luteal phase deficiency, as indicated by low serum progesterone values. Subjects 2 and 9 participated in low to moderate intensity aerobic activity greater than 7 hours per week and both experienced lower peak serum progesterone values as compared to other SC subjects, who exercised between 1 and 6 hours per week. The relationship between exercise frequency and menstrual dysfunction has been previously studied (Broocks et al. 1990, Bullen et al. 1985). While both Broocks et al. (1990) and Bullen et al. (1985) reported an effect of exercise on lowering luteal phase serum progesterone values, exercise intensity and duration in both studies far exceeded that of even the most frequent exercisers of the present investigation. The subjects in the Broocks et al. (1990) study ran 20 - 30



kilometers per week and were compared to sedentary controls. The subjects in the Bullen et al. (1985) study ran 4 miles per day and engaged in three and one half hours of moderate sports activity daily. The subjects experiencing disrupted menstrual cycles also lost  $4 \pm 0.3$  kg over the 5 week training period. The subjects in the present study exercising the most (Subjects 2 and 9) did so on average one to one and a half hours per day, without using running for activity, and without weight loss. Both Subject 2 and 9, however, had significantly lower serum peak progesterone levels than other SC subjects and both experienced luteal phase deficiency based on these progesterone values. These results suggest that women scoring highly on the EAT-26, thereby holding beliefs and attitudes consistent with those attributed to disordered eating, and who exercise on average 1 hour per day or more, may be more likely to experience menstrual cycle dysfunction than other normal weight subjects dieting to lose weight.

#### Limitations and Proposed Directions for Future Research

A limitation to this investigation was the inclusion of subjects who had a history of disrupted menstrual cycles. The results of this investigation may have been confounded by underlying menstrual cycle abnormalities not necessarily associated with dietary intake. The use of subjects with no

previous menstrual cycle abnormalities or who all had experienced only diet-related menstrual changes would have helped to ensure homogeneity of subjects across groups.

The effect of physiological, psychological, and nutritional stressors is a confounding variable which was not accounted for and which, therefore, served as a limitation. These stressors can have a direct impact on menstrual hormone patterns through increased corticotropin releasing hormone (CHR) secretion which is known to interfere with LH pulsatility (Barr et al. 1994). Through this and other still unknown mechanisms, stress may result in hypothalamic amenorrhea, anovulation, and increased estrogen production (Speroff et al. 1978). The use of an indicator of physiological, psychological, or nutritional stress in future investigations may provide further insight into menstrual cycle dysfunction in this group of normal weight dieters.

Dietary restraint was not measured in this investigation. The use of the Restraint Scale of the Three Factor Questionnaire has been used previously to identify subjects with high cognitive, and therefore dietary, restraint (Schweiger et al. 1992). This same questionnaire could be used with the present study design in the future to determine if cognitive restriction of dietary energy would effect menstrual hormone patterns in normal weight subjects. Dietary restraint, in particular, chronic inadequate energy intake, can be measured biochemically through identification of metabolic and

endocrine indicators of starvation. Measures of serum triiodothyronine ( $T_3$ ) or elevated beta-hydroxybutyric acid (BHBA) have been used previously to identify sub-optimal energy intake in anorectic and bulimic subjects (Pirke et al. 1985, Fichter et al. 1990). In conjunction with 24-hour LH secretory patterns, the measurement of  $T_3$  or BHBA in normal weight, restrictive dieters with luteal deficiency would be an important next step in determining the root of menstrual disturbances in this population. Serum samples from subjects participating in this investigation will be analyzed for  $T_3$  and cortisol by Janet Wojcik, a graduate student in Human Nutrition, Foods and Exercise at Virginia Tech. Corticotropin releasing hormone stimulates cortisol secretion and serum cortisol has been shown to be elevated in restrained eaters (Pirke et al. 1990). The results of this collaborative effort will provide more insight into the etiology of menstrual dysfunction in this rarely studied population.

Another important limitation to this research was the use of blood samples from only one menstrual cycle. Although previous investigations have also used serum hormone data from only one month while still attaining significant results (Pirke et al. 1987, Schweiger et al. 1992), the use of serum hormone data from multiple cycles would possibly provide more reliable results. It is suggested that in future research, hormone data from at least two menstrual cycles should be included. Finally, the analyses of the

results of this investigation were limited by a small power. However, although the sample consisted of just 14 subjects, this investigation represented the first to use blood assay in conjunction with current diet and activity records in a group of normal-weight, college-age, female dieters and high scorers on the EAT-26. The data presented here are intended to be pilot in nature and the basis from which future investigations may proceed.

Further investigations which explore the occurrence of menstrual cycle dysfunction among normal weight women who participate in weight loss practices are needed. Due to the limitations of the present investigation, it is suggested that similar research involving a larger sample size, measures of dietary restraint and psychological and physiological stress, and blood samples from at least two menstrual cycles could potentially enhance these findings. An investigation of specific nutrient deficiencies or excesses in normal weight women who are dieting to lose weight and the possible interplay with menstrual hormone status would be another avenue for future research and would involve more extensive dietary records than those implemented in this investigation. It has been shown that the use of a vegetarian diet for weight loss may result in a diminished amount of dietary fat and an increased intake of dietary fiber, both of which have been shown to effect estradiol concentrations. Although none of the subjects participating in this study were complete vegetarians, an investigation of menstrual hormone function

in normal weight women using vegetarianism for weight loss would be of interest.

### Conclusions

Drewnowski et al. (1994) found that as many as 86% of female college freshmen surveyed were dieting for weight loss. If menstrual cycle dysfunction is indeed associated with weight loss practices in normal weight college-age women, then a vast proportion of the female college population might experience subtle alterations in menstrual hormone status. Five out of 14 of subjects participating in this investigation experienced menstrual cycle dysfunction. This dysfunction occurred in the absence of significant weight loss or loss of body fat, excessive exercise, without significant caloric restriction, and independent of EAT-26 score. Previous investigations of menstrual cycle dysfunction in women with symptomatic scores on the EAT-26, relied on self-report of menstrual history for information. The present investigation assessed serum values of estradiol and progesterone in conjunction with current records of dietary intake, dieting behavior, exercise frequency, and self-reported menstrual periods in women with symptomatic scores on the EAT-26 and women simply dieting to lose weight.

The results of this investigation provide further evidence to suggest that normal weight women who diet to lose weight experience menstrual dysregulation which may be expressed as amenorrhea, irregular menses, luteal phase deficiency, and anovulation. While the root of this problem could not be determined through the analysis of the data presented here, one point is clear; Thirty six percent of a small sample of college age women who were dieting to lose weight had aberrant serum progesterone levels. Should these women continue to diet for weight loss despite being of normal weight, they may, in the future, be faced with the problem of diet-induced infertility. It is the hope of this author that the information presented here will encourage those who educate, counsel and treat women at reproductive endocrinology clinics to screen patients for dieting behavior and undisclosed eating disorders before proceeding with what may be unnecessary invasive, expensive, and dangerous techniques to promote fertility.

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## APPENDIX A

### DSM-IV CRITERIA FOR ANOREXIA NERVOSA AND BULIMIA NERVOSA

#### **Anorexia Nervosa**

- A. Refusal to maintain body weight at or above minimally normal weight for age and height (e.g., weight loss leading to maintenance of body weight less than 85% of that expected; or failure to make expected weight gain during period of growth, leading to body weight less than 85% of that expected).
- B. Increased fear of gaining weight or becoming fat, even though underweight.
- C. Disturbance in the way in which one's body weight or shape is experienced, undue influence of body weight or shape on self-evaluation, or denial of seriousness of current low body weight.
- D. In postmenarcheal females, amenorrhea, (ie., the absence of at least three consecutive menstrual cycles).

#### Subtypes

Restricting Type: during the current episode of anorexia nervosa the person has not regularly engaged in binge-eating or purging behavior (ie., self-induced vomiting or the misuse of laxatives, diuretics, or enemas).

Binge-Eating/Purging Type: during the current episode of anorexia nervosa, the person has regularly engaged in binge-eating or purging behavior (ie., self-induced vomiting or the misuse of laxatives, diuretics, or enemas).

#### **Bulimia Nervosa**

- A. Recurrent episodes of binge-eating. An episode of binge-eating is characterized by both of the following:
  - (1) eating in a discrete period of time (e.g., within any 2-hour period), an amount of food that is definitely



larger than most people would eat during a similar period of time and under similar circumstances.

(2) a sense of lack of control over eating during the episode (e.g., a feeling that one cannot stop eating or control what or how much one is eating).

- B. Recurrent inappropriate compensatory behavior in order to prevent weight gain, such as self-induced vomiting; misuse of laxatives, diuretics, enemas, or other medications; fasting; or excessive exercise.
- C. The binge-eating and inappropriate compensatory behaviors both occur, on average, at least twice a week for 3 months.
- D. Self-evaluation is unduly influenced by body shape and weight.
- E. The disturbance does not occur exclusively during episodes of Anorexia Nervosa.

#### Subtypes

Purging Type: During the current episode of bulimia nervosa, the person has regularly engaged in self-induced vomiting or the misuse of laxatives, diuretics, or enemas.

Nonpurging Type: during the current episode of bulimia nervosa, the person has used other inappropriate compensatory behaviors, such as fasting or excessive exercise, but has not regularly engaged in self-induced vomiting or the misuse of laxatives, diuretics, or enemas.

(American Psychiatric Association 1994)

APPENDIX B

FLYER

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Subjects needed for research project:

## **Weight Loss Practices and Women's Health**

Females, ages 19 - 25, who are currently dieting to lose weight or are concerned about weight gain or weight loss issues are invited to participate in this study. You must meet the following criteria:

- **Must not be using the Pill or other hormone-based medication**
- **Must not be pregnant nor plan to become pregnant prior to June 1995**

**Please call Michele Lewis in the Department of Human Nutrition and Foods at 231-7708 or at 552-8279 for further information about participation.**

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## APPENDIX C

### INFORMED CONSENT

#### **VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY Informed Consent for Participants of Investigative Projects**

This study, entitled, “Weight Loss Practices and Menstrual Cycle Function in College-Age Women” is being conducted by Michele D. Lewis, B.A., a Masters student in the Department of Human Nutrition and Foods at Virginia Tech and Dr. Elizabeth Thomas, Ph.D., R.D.

#### **I. The Purpose of This Research**

You are invited to participate in a study about the effects of weight loss practices and dieting on the function of the menstrual cycle in young women. This study involves experimentation for the purpose of assessing reproductive hormone levels as they relate to food intake and food related behaviors.

#### **II. Procedures**

If you choose to participate in this project, you will be asked to complete the Eating Attitudes Test (EAT - 26) and a personal interview asking for information about your food intake, beliefs and attitudes about your weight and weight loss, your menstrual history, and food-related behaviors. You will be required to have 10 weekly blood draws of 15 ml of whole blood. This is equivalent to 1 Tbs of blood per week. The total amount of blood collected throughout the study will be 150 ml (10 Tbs), less than one third the amount taken at a typical blood donation. Please make note of these and other requirements for participating which are listed in Section IX.

#### **III. Risks**

No harm to you is expected to result from any of these activities. However, you may experience some emotional distress when completing the EAT - 26 and the during the preliminary interview. In order to minimize any discomfort or distress you may feel, all interviews will be conducted confidentially and all participants will be provided with a list of student support service telephone numbers. You may also experience some distress related to the weekly blood samples you will be required to provide. To minimize your risk or discomfort, a trained phlebotomist will be conducting the blood draws. Juice and bagels will be provided after the blood draws.

#### **IV. Benefits of this Project**

Your participation in this project will provide the following information that may be helpful:

- the presence or absence of menstrual cycle abnormalities
- determination of presence or absence of ovulation
- free nutritional assessment and dietary counseling

No guarantee of benefits has been made to encourage you to participate. At the completion of this study, Michele D. Lewis will consult with you individually to discuss the results of your personal tests.

## **V. Extent of Anonymity and Confidentiality**

The results of this study will be kept strictly confidential. At no time will the principle investigator release the results of the study to anyone other than the individuals working on the project without your written consent. The information you provide will have your name removed and only a subject number will identify you during analysis and any written reports of the research.

## **VI. Compensation**

For participation in the project you will receive 1 hour of undergraduate research credit. You will also receive a specially designed "Fitness Package" upon completion of this study. This "Fitness Package" will include a complete biochemical profile involving the analysis of one of your blood samples, accurate measurement of body fat, measurement of basal metabolic rate, and a one day pass to the University Weight Club in the University Mall. Other incentives will be provided throughout the course of the study.

## **VII. Freedom to Withdraw**

Your participation is voluntary and you are free to withdraw from this study at any time without penalty. There may be circumstances under which the investigators may determine that you should not continue as a subject of this project. These circumstances include chronic failure to fulfill any one of the responsibilities outlined in Section IX.

## **VIII. Approval of Research**

This research project has been approved, as required, by the Institutional Review Board for projects involving Human Subjects at Virginia Polytechnic Institute and State University and by the Department of Human Nutrition and Foods.

## **IX. Subject's Responsibilities**

I know of no reason that I cannot participate in this study. I have the following responsibilities:

1. Participation in an unstructured preliminary interview focused upon menstrual history, food intake, and food related behaviors.
2. Participation in an orientation on the use of the daily food intake and food-related behavior records and the proper procedure for measuring basal body temperature and assessing menstrual status.
3. The daily completion of food intake and food-related behavior records, daily body temperature recording and the keeping of menstrual cycle records.
4. The weekly donation of up to 15 ml of blood for use in enzyme-based assays of serum estrogen and progesterone concentrations. Lab times will be scheduled in advance and I will fast for 12 hours prior to each blood draw.
5. Inform principle investigator of any changes in medications or if I am pregnant.
6. Inform principle investigator if illness or circumstances prevent my attendance to the lab within 24 hours of my scheduled time and will at that time reschedule.
7. Participation in an unstructured exit interview at the end of the project.

---

Signature of Participant

Date

**X. Subject's Permission (For subject to keep)**

I have read and understand the informed consent and conditions of this project. I have had all of my questions answered. I hereby acknowledge the above and give my voluntary consent for participation in this project. If I participate, I may withdraw at any time without penalty. I agree to abide by the rules of this project.

Should I have any questions about this research or its conduct, I will contact:

Michele D. Lewis - Principle Investigator                      552-8279 (h) or 231-7708 (w)

Dr. Elizabeth Thomas - Faculty Advisor                      231-8763

Ernest R. Stout    231-9359  
Chair, IRB  
Research Division

## APPENDIX D

### EATING DISORDER INFORMATION SHEET

#### **Information About The Eating Disorders**

Because of the nature of this investigation and the high prevalence of clinical eating disorders among women of college age, it is our obligation to inform you of the support services available to Virginia Tech students in the event that you or someone you know should need assistance.

The eating disorders, Anorexia Nervosa, Bulimia Nervosa, and Binge Eating Disorder, are not about weight loss or weight maintenance. Each disorder involves an intricate relationship with food which goes beyond eating for sustenance. The young woman with an eating disorder may begin her journey into self-destructive behavior with the intent to simply “lose a few pounds”. Underlying psychological and emotional stresses, coupled with what is sometimes referred to as a “spiritual emptiness”, turns simple weight loss into an obsessive desire for perfection and control over what may be a very chaotic life. The woman with Anorexia Nervosa is able to use this control to restrict her food intake and thus maintain an outward appearance of physical perfection. The Bulimic, unable to control her food intake, finds herself bingeing on large amounts of food and then ridding herself of the food through self-induced vomiting or laxative abuse. The woman with binge eating disorder, like the bulimic, consumes large amounts of food when she is bored, tired, angry, or lonely, however, she does not purge.

It is quite common for young women to engage in behaviors associated with the eating disorders in the quest for thinner bodies. Many women on occasion limit their food intake or take diet pills when they feel they must shed some weight. The difference between these women and those with clinical eating disorders lies in the frequency and the severity of these behaviors and the obsessive fear of becoming overweight.

Should you or someone you know need assistance, please contact one of the following student focused support services:

- Center for Family Services 231-7201
- Psychological Services Center 231-6914
- University Counseling Services 231-6557
- Campus Ministries 231-3787

APPENDIX E

EATING ATTITUDES TEST

Subject Code \_\_\_\_\_

Date \_\_\_\_\_

Please place an (X) under the column which applies best to each of the numbered statements. All of the results will be strictly confidential. Most of the questions directly relate to food or eating although other types of questions have been included. Please answer each question carefully. Thank you.

- | A                        | VO                       | O                        | S                        | R                        | N                        |  |
|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--|
| <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | 1. Am terrified about being overweight.  |
| <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | 2. Avoid eating when I am hungry.  |
| <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | 3. Find myself preoccupied with food   |
| <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | 4. Have gone on eating binges where I feel I may not be able to stop.                            |
| <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | 5. Cut my food into small pieces.  |
| <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | 6. Aware of the calorie content of the foods I eat.  |
| <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | 7. Particularly avoid foods with a high carbohydrate content (e.g. bread, rice, potatoes, etc.). |
| <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | 8. Feel that others would prefer if I ate more.  |
| <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | 9. Vomit after I have eaten.   |
| <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | 10. Feel extremely guilty after I have eaten.  |
| <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | 11. Am preoccupied with a desire to be thinner.  |
| <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | 12. Think about burning up calories when I exercise.   |
| <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | 13. Other people think that I am too thin.   |
| <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | 14. Am preoccupied with the thought of having fat on my body.                                    |
| <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | 15. Take longer than others to eat my meals.   |
| <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | 16. Avoid food with sugar in them.   |
| <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | 17. Eat diet foods.  |
| <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | 18. Feel that food controls my life.   |
| <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | 19. Display self-control around food.  |
| <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | 20. Feel that others pressure me to eat.   |
| <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | 21. Give too much time and thought to food.  |
| <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | 22. Feel uncomfortable after eating sweets.  |
| <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | 23. Engage in dieting behavior.  |
| <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | 24. Like my stomach to be empty.   |
| <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | 25. Enjoy trying new rich foods.   |
| <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | 26. Have the impulse to vomit after meals.   |

APPENDIX F

INITIAL INTERVIEW

**General Information**

Date \_\_\_\_\_  
Name \_\_\_\_\_ Code Number \_\_\_\_\_  
Current Address \_\_\_\_\_  
Permanent Address \_\_\_\_\_  
Current Telephone \_\_\_\_\_ Permanent Telephone \_\_\_\_\_  
Social Security Number \_\_\_\_\_  
Date of Birth \_\_\_\_\_  
Education Level  
     Fresh     Soph     Jr     Sr     Grad

**Weight History**

Current Weight \_\_\_\_\_  
Current Height \_\_\_\_\_  
Highest Weight Since Age 12 \_\_\_\_\_  
Lowest Weight Since Age 12 \_\_\_\_\_  
How long did you remain at your lowest weight? \_\_\_\_\_  
Desired Weight \_\_\_\_\_

At your current weight do you feel that you are:

Extremely Thin	Somewhat Thin	Normal Weight	Somewhat Overweight	Extremely Overweight
1	2	3	4	5

How much does a two-pound weight gain affect your feelings about yourself?

Extremely Negative	Somewhat Negative	Neutral	Somewhat Positive	Not at All Negative
1	2	3	4	5

How much does a two-pound weight loss affect your feelings about yourself?

Extremely Positive	Somewhat Positive	Neutral	Somewhat Negative	Not at All Positive
1	2	3	4	5



How dissatisfied are you with the way your body is proportioned?

Extremely Dissatisfied	Very Dissatisfied	Moderately Dissatisfied	Slightly Dissatisfied	Not at All Dissatisfied
1	2	3	4	5

How often do you weigh or measure your body size?

More than daily                       Daily  
 More than weekly                       Weekly  
 Less than monthly                       Monthly

**Dieting History**

Have you ever been on a diet?  Yes  No

At what age did you begin dieting due to concern over your body size? \_\_\_\_\_

Please check your preferred way of dieting:

Skip meals                                       Reduce portions  
 Fasting     Go on "fad diets"  
 Restrict carbohydrates                       Reduce calories  
 Restrict sweets                                       Other (please specify)  
 Restrict fats

Have you ever had an episode of eating a large amount of food in a short space of time (an eating binge)?  Yes  No

Circle all that apply:

I consume a large amounts of food.	Never	Rarely	Sometimes	Often	Always
I eat very rapidly.	Never	Rarely	Sometimes	Often	Always
I feel out of control when I eat.	Never	Rarely	Sometimes	Often	Always
I feel miserable and annoyed after a binge.	Never	Rarely	Sometimes	Often	Always
I get uncontrollable urges to eat and eat until I feel physically ill.	Never	Rarely	Sometimes	Often	Always
I binge eat in private.	Never	Rarely	Sometimes	Often	Always

Have you ever vomited food after eating in order to get rid of the food eaten?  Yes  No

Are you currently using self-induced vomiting as a means to get rid of food eaten?  Yes  No

Have you ever used laxatives to control your weight or to get rid of food?  Yes  No

Are you currently using laxatives to control your weight? \_\_\_\_\_ Yes \_\_\_\_\_ No

During the entire last month, what is the average frequency with which you have engaged in the following behaviors? (Check one for each behavior)

	Never	Once a Month	More than once a month	Once a Week	Several times a week	Once a day	More than once a day
Binge eating							
Vomiting							
Laxative use							
Use of diet pills							
Use of enemas							
Exercise for weight loss							
Fasting for an entire day							
Greatly decreased intake							

**Menstrual History**

At what age did you start your period? \_\_\_\_\_

How would you describe the regularity of your period in general?

- \_\_\_\_\_ Very regular
- \_\_\_\_\_ Somewhat regular
- \_\_\_\_\_ Somewhat irregular
- \_\_\_\_\_ Very irregular

Have you noticed any changes in your menstrual cycle since you began dieting?

\_\_\_\_\_ Yes \_\_\_\_\_ No

If yes, please describe. \_\_\_\_\_

Have you ever stopped menstruating for a period of 3 or more months?

\_\_\_\_\_ Yes \_\_\_\_\_ No

Are you currently menstruating?

\_\_\_\_\_ Yes \_\_\_\_\_ No

Are you currently using birth control pills?

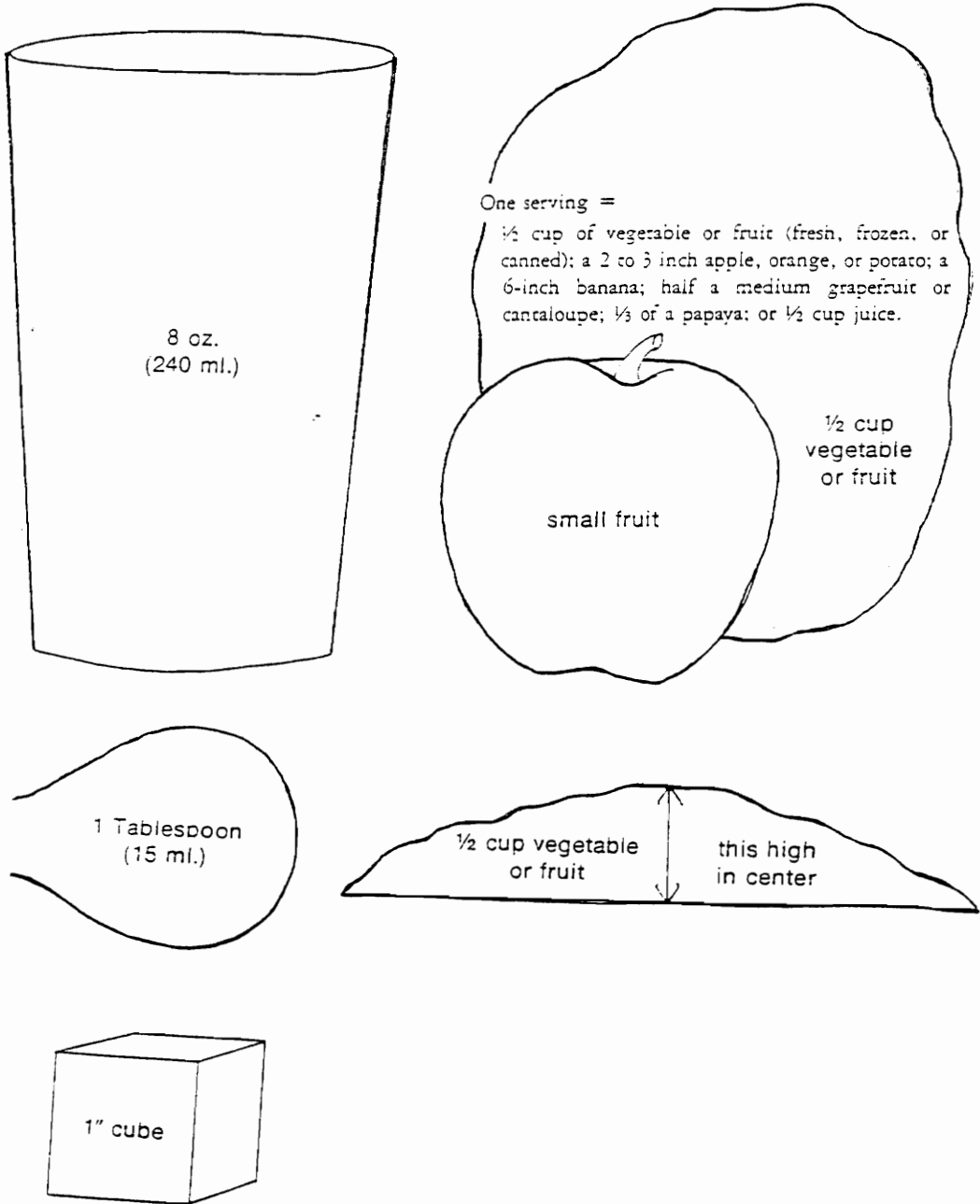
\_\_\_\_\_ Yes \_\_\_\_\_ No



# APPENDIX H

## SERVING SIZE ESTIMATES

### RECOMMENDED SERVING SIZES



## APPENDIX I

### SUBSTITUTIONS MADE IN DIET RECORD ANALYSIS

Subject Food Selection	Nutritionist III Alternative
Owen's mini loaf (132 kcal)	Cracked wheat bread (2 slices)
Girl Scout cookies or non-descript cookies	Plain mix cookies
Non-fat frozen yogurt	Non-fat vanilla yogurt
Harvest (veggie) burger	Fried tofu (3 oz)
Cauliflower	Broccoli
Lemon poppyseed muffin	Blueberry muffin
Nutri-grain bar	Fruit bar
Grapefruit whole	Grapefruit juice
Chocolate brownie	Dark chocolate cake
Diet drink - i.e. Slim Fast	Vanilla shake low-fat
Non-descript cereal	Kellogg's Corn Flakes
Low-fat granola bar	Granola Bar (90 kcal)
Low-fat breakfast bar	Granola Bar (90 kcal)
Non-descript bread and lo-cal bread	Bread- white, soft
McLean Deluxe	Hamburger-plain
Non-descript mixed vegetables	Mixed vegetables - canned or Mixed vegetables - frozen boiled
Non-descript mixed fruit	Fruit cocktail canned in water
Fat-free granola	1/2 serving of regular granola

APPENDIX J

DAILY RECORD OF FOOD-RELATED BEHAVIORS  
AND EXERCISE

In the table below, please place an “X” in the column to the left of any food-related behavior or exercise in which you engage on each day of this record. Please be sure to note specific details where indicated in the table.

Date \_\_\_\_\_

Subject Code \_\_\_\_\_

	<b>I exercised in order to improve or maintain my fitness level. Type &amp; duration of each exercise session:</b>
	<b>I consumed an excessive amount of food at one time.</b>
	<b>I used laxatives after overeating. Number of laxatives taken this day:</b>
	<b>I skipped a meal because I was too busy or I forgot to eat. Number of meals skipped this day:</b>
	<b>I used “diet pills” as a means to lose weight Number of diet pills taken this day:</b>
	<b>I counted the fat grams in the food I ate Number of fat grams consumed this day:</b>
	<b>I caused myself to vomit after overeating Number of times vomited this day:</b>
	<b>I skipped a meal in order to lose weight Number of meals skipped this day:</b>
	<b>I counted the calories that I consumed Number of calories consumed this day:</b>
	<b>I skipped dessert in order to cut down on my calorie consumption.</b>
	<b>I used a weight loss supplement (i.e. Ultra Slim Fast) to lose weight. Number of meals replaced with supplement this day:</b>
	<b>I exercised to compensate for eating too much. Type &amp; duration of each exercise session:</b>

Comments:

## APPENDIX K

### DAILY RECORD OF MENSTRUAL CYCLE

In the space provided on the attached calendar, please record the appropriate **letters** associated with your menstrual cycle function for **each specific day**. Please use the letters listed in the key below.

#### Key for Menstrual Cycle Function

<b>S</b>	Started Period
<b>M</b>	Currently Menstruating
<b>N</b>	Not Currently Menstruating
<b>F</b>	First Day of No Bleeding After Period
<b>L</b>	Light Flow (2-3 tampon / pad changes per day)
<b>H</b>	Heavy Flow (more than 3 tampon / pad changes per day)
<b>SBP</b>	Spotting <b>B</b> etween <b>P</b> eriods
<b>SDP</b>	Spotting <b>D</b> uring <b>P</b> eriods (1-2 tampon / pad changes per day)

For example, for each day that you are not menstruating (i.e. not bleeding), place an “N” in the appropriate spaces on the calendar. When you begin your period, use the letter “S” and the letter “M” to indicate that you started your period and that you are currently menstruating. If you are menstruating when you begin this project, place the letter “M” in the space for the date that you begin recording. Also, please note the **rate of flow** you are experiencing (i.e. heavy, light, or spotting) for each day that you are menstruating.

**FEBRUARY**

<b>Sunday</b>	<b>Monday</b>	<b>Tuesday</b>	<b>Wednesday</b>	<b>Thursday</b>	<b>Friday</b>	<b>Saturday</b>
			1	2	3	4
5	6	7	8	9	10	11
12	13	14	15	16	17	18
19	20	21	22	23	24	25
26	27	28				

**MARCH**

<b>Sunday</b>	<b>Monday</b>	<b>Tuesday</b>	<b>Wednesday</b>	<b>Thursday</b>	<b>Friday</b>	<b>Saturday</b>
			1	2	3	4
5	6	7	8	9	10	11
12	13	14	15	16	17	18
19	20	21	22	23	24	25
26	27	28	29	30	31	

**APRIL**

<b>Sunday</b>	<b>Monday</b>	<b>Tuesday</b>	<b>Wednesday</b>	<b>Thursday</b>	<b>Friday</b>	<b>Saturday</b>
						1
2	3	4	5	6	7	8
9	10	11	12	13	14	15
16	17	18	19	20	21	22
23	24	25	26	27	28	29



## APPENDIX L

### Post-Study Subject Survey

Please complete the following survey and return it by mail in the attached addressed envelope. This survey is **anonymous** and simply serves to assist me in analyzing the data you have provided and to help me assess the validity of the results I obtain. Neither your name nor number is associated with this information and you may chose to type your responses. Thank you again for all your help.

---

---

1. Did you complete your Food Intake records accurately and honestly?

YES

NO

2. Did you complete your Food Related Behavior records accurately and honestly?

YES

NO

3. Did you complete Menstrual Cycle and Temperature records accurately and honestly?

YES

NO

4. If you answered NO to any of the above questions, please provide a brief explanation.

---

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5. I am interested in learning what was the most important motivating factor which led you to remain with this study for its entirety. With any study involving long-term blood work, there is a varying degree of non-compliance or drop-outs. I feel very fortunate that the fourteen of you who began the study, stayed until its completion (sore arms and all). Please check all that apply and comment on this issue.

\_\_\_\_\_ Janet Rinehart's skill in drawing blood

\_\_\_\_\_ Personal interest in the results

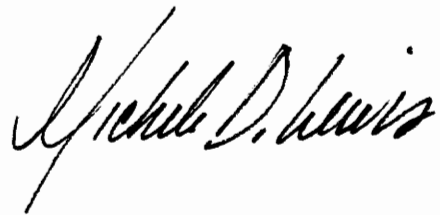
\_\_\_\_\_ Michele Lewis' role as facilitator/bagel chef

\_\_\_\_\_ Communication with other subjects

\_\_\_\_\_ Other (Please specify)

## VITA

Michele Ann Dow was born in Chula Vista, California on October 17, 1966. In June, 1988, she graduated *cum dignitate* with a Bachelor's degree in Psychology from the University of the South. In 1989, she married Eric Parkfield Lewis. Her employment experience has been in early childhood education and in marketing with the National Aquarium in Baltimore. Her graduate work began in 1993 when she enrolled in a Master's degree program with the Department of Human Nutrition and Foods at Virginia Tech. Her research interests include the physiological and psychological impact of sub-clinical eating disorders and moderate nutrient deficiencies. After graduation, and the completion of a dietetic internship program, she hopes to begin a career which encompasses nutrition counseling, clinical management, and eventually, entrepreneurship.

A handwritten signature in black ink that reads "Michele D. Lewis". The signature is written in a cursive style with a large, sweeping initial 'M'.