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Effects of Exogenous Female Sex Hormones on Food Intake, Macronutrients and Body Weight in the Ovariectomized Postbreeder Female Rat.

Jan Barton Hamilton
Louisiana State University and Agricultural & Mechanical College

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EFFECTS OF EXOGENOUS FEMALE SEX HORMONES ON FOOD INTAKE, MACRONUTRIENTS AND BODY WEIGHT IN THE OVARIECTOMIZED POSTBREEDER FEMALE RAT

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
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in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The School of Human Ecology

by
Jan B. Hamilton
B.S., Texas Tech University, 1963
M.S., Texas Tech University, 1992
December 1995

DEDICATED TO MY

LOVING HUSBAND WHO NEVER STOPPED ENCOURAGING ME

AND TO DOC AND IRENE PENNINGTON

and to every individual who ever wanted to change body weight or metabolism during the lifespan

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TERMS AND ABBREVIATIONS

TERMS

Menopause:	cessation of the menses
Perimenopause:	begins at age 35 with declining estrogen levels
Postmenopause:	years beyond menopause
Postmenopausal:	no menstrual period for 12 months
Estrogen:	female sex hormone dominant in follicular phase of menstruation
Progesterone:	female sex hormone dominant in luteal phase of menstruation
Ovariectomized:	surgical removal of the ovaries
Sham:	surgery without the removal of the ovaries
Luteal phase:	12-14 days following ovulation
Follicular phase:	12-14 days prior to ovulation
Estrus:	recurring period of maximum sexual receptivity in most female mammals
Diestrus	interval between periods of estrus
Proestrus:	period immediately following estrus
Metestrus:	period following estrus and preceding diestrus
Negative allesthesia:	hunger or appetite
Hyperphagia:	hunger or increase in appetite and food consumption
Hypophagia:	satiety or decrease in appetite and food consumption
Hyperplasia:	build-up of the endometrial lining

ABBREVIATIONS

BW:	Body weight
OV:	Ovariectomized
S:	Sham surgery
E:	Estrogen or Estradiol
P:	Progesterone
H	Hormone
PRO:	Protein
CHO:	Carbohydrate
C:	Chocolate
FAT:	Fat
g:	Grams
BMI:	Body Mass Index: weight in kilograms divided by height in meters (squared)
kcal:	Kilocalories
HDL:	High density lipoproteins
Chol:	Cholesterol
LH:	Luteinizing hormone
FSH:	Follicle stimulating hormone
GnRH:	Gonadotropin-releasing hormone
HRT:	Hormone replacement therapy
CHD:	Coronary heart disease

ABSTRACT

Hormone replacement therapy (HRT) is chosen by a growing segment of the postmenopausal population. Mid-life body weight gain is perceived to increase further with exogenous HRT. To examine hormonal effects on caloric intake (CI), carbohydrate (CHO), FAT, protein (PRO), chocolate and body weight (BW) in a female model, Sprague-Dawley postbreeder (n=55) rats (10 mos., 10 litters of pups) were ovariectomized (OV) and implanted with 17 β estradiol (E) and/or progesterone (P), or placebo in three separate studies (phases) of 10 days each. Uterine weights (p=.0001) and radioimmunoassay confirmed hormonal bioactivity. The sham (S) group with placebo implant was used for comparison.

In phase I, 3 food cups containing CHO, FAT, and PRO were presented ad libitum to all treatments. Estrogen decreased the rate of body weight gain (p=.001) compared to OV, P, and S with no significant differences in caloric intake (trend of estrogen p=.052). In phase II, all except S received 4 food cups; 2 CHO choices, (sweet, AIN 76 and nonsweet AIN 93), FAT and PRO. The body weight of the P and S groups compared to OV (p=.009) in phase II adapted and did not continue to increase. OV produced a carbohydrate appetite for both SW & NSW (p=.007), E&P chose 3 times more SW than NSW (p=.001). For phase III 4 caloric levels of chocolate were added (except for S). Chocolate was consumed at 40% to 53% of total caloric intake with or without HRT with reduced nutrient dense macronutrient consumption. Thus access to chocolate eliminated both the reduced rate of weight gain caused by E (phase I) and the body weight adaptation by P in phase II. Variations in % fat intake (40% to 60%) did not result in treatment differences in body composition

(p=.0945). The OV group which consumed the most calories from carbohydrate (p=.001), gained the most overall BW (p=.001). The rats consuming the most fat (S 57%) gained the least amount of body weight (p=.001). Caloric conversion ratios (weight gain/by caloric intake x 1000) varied among treatments (p=.003). Additional research on the metabolism of the postmenopausal female taking hormone replacement therapy is needed.

CHAPTER 1: INTRODUCTION

There are 470 million women aged 50 years and older living in the world today (World Health Organization [WHO]: World Health Statistics Annual, 1990). These women are older than the average age of natural menopause throughout recorded history. Only 50 years ago, even in developed countries the average woman did not live to be 50 years of age. On a global basis, there are large populations of women for whom the risks of estrogen deficiency are undefined. The largest potential benefit of estrogen replacement therapy is the prevention of heart disease, the major cause of death in the female. In countries with a relatively high risk of heart disease, current studies suggest that estrogen reduces that risk by 50% (WHO, 1990). Due to recently reported clinical trials showing the cardioprotective effects of hormone replacement therapy, a large number of women in the USA and other developed countries may be prescribed extended estrogen replacement therapy (HRT) to reduce cardiovascular disease (The Writing Group for the PEPI Trial, 1995). Very little is known about the effects of exogenous hormones on energy balance in women. The common perception is that they may increase body weight (personal interviews with OB/GYN physicians).

Between puberty and menopause, serum estrogen and progesterone levels vary throughout the menstrual cycle. The follicular phase is estrogen dominant with high concentrations of estrogen and low progesterone levels. During the luteal phase, the progesterone levels rise to a degree that neither hormone is dominant. During pregnancy, levels of estrogen and progesterone rise steadily until they reach a plateau weeks before delivery. Circulating estrogen levels begin to decline in the perimenopausal years. At menopause,

the level of estradiol, produced primarily by the ovary, falls and is replaced by a less active estrogen, estrone, produced primarily by conversion of androstendione in adipose tissue. After the menopause there is little further decrease in endogenous estrogens with advancing age. Progesterone appears to decline at the same rate as estrogens. There is a widespread perception among women that this signal of menopause is responsible for weight gain after age 50. This belief is held both by women who are already overweight when they reach menopause, as well as those whose weight is normal prior to menopause.

A recent report by Willett, et al., (1995) at the Nutrition Center at Harvard University School of Public Health is being debated for validity. They report that obesity is a major risk factor for a variety of medical conditions: cardiovascular disease, hypertension, diabetes mellitus, reproductive cancers, arthritis, and kidney disease, and that if menopause is found to accelerate weight gain among women who are already obese and at medical risk, then steps need to be taken prior to the menopause to minimize its impact on weight gain among those who are most vulnerable. Body weight maintenance to reduce risks is prudent however, often this kind of reporting can create negative health behaviors. The rates of anorexia nervosa and bulimia are increasing. We see the impact of this concept in the fact that $33 billion is spent yearly on weight control in this country (Atkinson,1990). Low fat diets and body weight reduction in the female have become national obsessions.

Therefore, the issue of whether menopause, age or hormone replacement therapy provokes weight gain must be examined critically. It is important to know what effect hormone replacement has on food choices and

weight gain. Due to the current emphasis on thinness and low fat diets (Ley, et al, 1992), the female often resists any involvement in exogenous agents that could cause body weight gain regardless of the potential positive effects.

Some reports in the early 1990s on the effect of obesity on cardiovascular risk in women (n=115,886) show that after adjusting for age and smoking, the risk is 3.3 times higher in women with a body mass index (BMI) of >29.0 compared to a BMI of <21.0 for non obese women. These authors have concluded that the risk of coronary heart disease (CHD) in middle-aged women increases even among those who were only moderately overweight (Manson, Willett, & Stampfer, 1995). The priority of body weight seems to supersede all other concerns. Therefore, if the female feels that hormone replacement can cause body weight gain she may decline its use or fail to comply with the physician's prescription. Recent reports have shown that compliance drops to 8% after three years and that 50% of the females given the prescription for hormone replacement therapy (HRT) never have the prescription filled (Lerner, 1995).

Health implications surrounding body weight and HRT are also relevant because it is known that obesity increases the risk of breast cancer in postmenopausal women (Vatten, & Kvinnsland, 1990; Sellers, et al., 1992; Troisi, 1995). The dilemma of taking HRT is compounded if the perception also exists that the protective effect will be offset by the increased weight gain. Menopause is thought to be associated with a shift to upper body adiposity, which may be related to increased risk for cardiovascular disease, diabetes mellitus and breast cancer. Endometrial cancer has been strongly associated with obesity in postmenopausal women (Folsom, et. al.,1989). Unlike breast

cancer, it is linked to the amount rather than the distribution of fat stores. The issue of whether hormone replacement therapy initiates additional body weight gain should be scientifically measured and reported. At present, reports are not conclusive.

The positive effects of HRT are evident. However a recent report on the negative side effects of hormone replacement therapy stated that 50% of the women for whom hormone replacement medication had been prescribed had discontinued taking their medication in under 1 year. Earlier publications have cited a wide range of compliance problems, and the overall rate of discontinuation is surprisingly high. Among the list of negative side effects are persistent bleeding complications, severe mood changes and lack of adaptation and response to manipulation of therapy programs. Body weight gain and an increase in abdominal fat and enlarged breasts are often reported to physicians (Lerner, 1995). Many of the early complaints occurred when 10 mg of progesterone was the accepted dosage to maximally decrease the risk of reproductive cancer. Current Food and Drug Administration (FDA) clinical trials are using 2.5 continuous or 5 mg cyclically of progesterone combined with .625 mg of estrogen. The 10 mg dosage is no longer recommended. The newest combined levels are being tested for effectiveness against osteoporosis, heart disease with maximum endometrial protection. These trials will provide the "Guidance of Clinical Evaluation of Combination Estrogen/Progestin-Containing Drug Products Used for Hormone Replacement Therapy of Postmenopausal Women." The earliest reports by leaders in the field, state that in clinical trials the "new" drugs should (a) initially compare the rate of endometrial hyperplasia in the treated group to that in a

placebo group, (b) report the lowest progestin dose which is protective against endometrial hyperplasia, (c) establish the minimum effective dose of a specific progestin for any given dose of a specific estrogen (Grady & Cummings: 1995, Andrews, 1995, Gambrell, 1995, FDA HRT Working Group, 1995). Perhaps these new guidelines will correct earlier misconceptions, decrease negative physiological side effects, and ultimately increase compliance rates. However, when the perception of increased body weight is one of those side effects, the problem is often greater to overcome. More than vanity is at stake. According to the National Institute of Health, up to one third of all Americans are overweight, and they spend more than $30 billion per year in their efforts to trim the fat (Barinaga, 1995).

The current emphasis on dietary fat intake has created a national paranoia regarding anything that might trigger a fat appetite. Dietary fat intake may not be as strongly linked to a number of nutrition-related disorders, including obesity, heart disease and cancer, as earlier believed. The consumption of fat may be related to its palatability, high energy density, and ultimately positive physiological effects. Additional research is needed to understand how the sensory qualities of fat and individual differences in preferences for dietary fat influence human food intake and body composition and health related implications (Rolls, 1992). Changes in food intake during aging may also change the nutrient intake patterns related to the process of aging, not debilitating diseases (Kimura, 1992). Is there a chance that the appetite can be trusted and that moderate amounts of fat intake daily could again be considered a healthy choice? Is there hope that compliance to a

cardiovascular regime would be followed if women knew the body weight consequences of HRT? This author believes more information is needed.

This study was designed to examine hormonal effects on macronutrient food choices reflected in caloric intake and body weight. The metabolic implications within an aging animal model in a controlled environment may provide new information with potential implications for the mature human female. The purpose of this study was to examine patterns of caloric intake, macronutrient composition, and body weight gain in the female rat model (postbreeder) as affected by exogenous 17 ß estradiol (E) and/or progesterone (P), following ovariectomy. These are the hormones most often prescribed by physicians to treat menopausal symptoms. It is hoped that the study will yield data and provide new information to help understand how ingestive behavior, specific appetites, hunger motivation and body weight vary in relation to female sex hormones, following hysterectomy or natural menopause in mature women. This research design provides a controlled method of examining the effects of female sex hormones E, E&P, and P on food intake and body weight through administration of exogenous estrogen and progesterone in the retired breeder female rat(s) following ovariectomy.

PURPOSE AND OBJECTIVES

The overall purpose is to identify and quantify the effects of exogenous female sex hormones E, E&P, and P on food intake in ovariectomized postbreeder female rats. The specific objectives of the project are to:

1. Evaluate the effects of female sex hormones E, E&P, and P on total caloric intake and body weight gain.

2. Measure the effects of female sex hormones E, E&P, and P on nutrient composition (fats, carbohydrates [sweet and nonsweet] and proteins) of ad libitum food intake.
3. Measure the effects of female sex hormones E, E&P, and P on food choices of chocolate with high fat/high sugar, high fat/low sugar, low fat/high sugar and low fat/low sugar content.
4. Measure bioactivity of the hormones by assessing serum hormone levels of E, P and testosterone and measure cholesterol, HDL cholesterol, organ weights, fat content of livers and total body composition.

CHAPTER 2: REVIEW OF LITERATURE

Human health is a complex and multidetermined issue, influenced by a wide variety of factors: physiological, biochemical, nutritional, psychological, environmental, and social (Rodin & Salovey, 1989). Women's health is a priority of the 21st century. A number of health concerns are unique to women. Even for health issues that affect both women and men, most research has been limited to male subjects, leaving a large gap in our knowledge base concerning women's health.

The 1988 Surgeon General's Report on Nutrition and Health reports obesity to be the nation's number one nutritional problem (Public Health Service, 1988). The Healthy People 2000 (U.S. Printing Office, 1990) document reports that for 2 out of 3 Americans who neither smoke nor drink, eating patterns may shape their long-term health prospects more than any other choice. Due to increased longevity, the average U.S. woman will spend one-third of her life as a postmenopausal individual (Greendale & Judd, 1993). Recent census data from the U.S. indicates that in the 1990s the number of women above the age of 50 years will approach 40 million, and that a 50-year-old has a 30 year projected life-span. The quality of life during these years has been shown to be improved through hormone replacement therapy. Recently the results of the Postmenopausal Estrogen/Progestin Interventions (PEPI) study were reported revealing cardioprotective effects of hormone replacement therapy (The PEPI Writing Group, 1995). However, due to misconceptions regarding its implications to body weight gain many women are not choosing to take hormone replacement therapy and are therefore

depriving themselves of the advantages provided by its cardiovascular protective effects (Barrett-Conner, Bush, 1991).

MENOPAUSE AND POSTMENOPAUSAL THERAPY

In the perimenopausal woman, estrogen and progesterone levels decrease and luteinizing hormone (LH) and follicle stimulating hormone (FSH) become elevated, indicating the approaching menopause. The ovarian follicles grow in response to FSH. The estrogen produced in turn decreases delivery of gonadotropin-releasing hormone (GnRH). The decrease of GnRH then suppresses FSH and LH. As the ovaries age during natural menopause, the follicle store is depleted and estrogen production declines (Block, et al, 1952). In postmenopause, the depleted follicular status is generally the same regardless of the age at which menopause occurs. Without estrogen feedback, GnRH increases and, thus, FSH and LH rise substantially (Utian, 1978). Levels of FSH increase 10-20 times and LH three times within 1 to 3 years after menopause. These high levels are conclusive proof of menopause for a woman of menopausal age. In the postmenopausal woman, the mean daily level of estrogen production falls from 50-500 μg/d to 5-10 μg/d (for men, estrogen levels are 2 to 25 μg/day, equal to the urinary estrogens of a woman during the first week of her menstrual cycle). These estrogens result from the conversion of adrenal steroids (such as androstenedione) to estrone and estradiol. Levels of androstenedione post-menopause, however, are only half those prior to menopause. Testosterone levels do not decline significantly and may even rise in the postmenopausal woman (Zichella, 1993).

Symptomatic menopausal women who experience hot flashes, night sweats, sleep disturbances, mood disturbances, impairment of memory and

concentration, and muscle and joint pains with or without lower genital tract atrophy may actively seek hormone replacement therapy (Marsh & Whitehead, 1992). The safety and efficacy of a daily combination of micronized estradiol (E_2) (0.7-1.05 mg) and progesterone (200-300 mg) were evaluated in ten menopausal women with moderate to severe vasomotor symptoms and/or vaginal atrophy over a 12-month study interval. Resulting evidence revealed symptomatic improvement, minimal side effects, an improved lipid profile, and amenorrhea without endometrial proliferation or hyperplasia in menopausal women (Hargrove, et. al., 1989). The micronized form of progesterone is extracted from a natural source such as soybean or yams and was shown in the PEPI investigation to be the most effective form of progesterone (The PEPI Writing Group, 1995). Currently, the most widely used exogenous female sex hormones by postmenopausal women are Premarin® and Provera®. The decision to utilize these or other exogenous forms of exogenous female sex hormones generally occurs during the perimenopausal period when symptoms can intensify and alter the quality of life as previously mentioned.

Physiologically following the perimenopause, menstrual cycles cease and the secretion of steroid hormones and gonadotropin changes. Postmenopausal levels of circulating estradiol are lower than levels seen during the menstrual cycle (Judd, Judd, & Lucas, 1974). The major source of estradiol becomes the peripheral conversion of estrone and testosterone. In contrast to the ratio seen in premenopausal women, postmenopausal estrone levels are higher than estradiol levels. Postmenopausal estrone is also derived from the peripheral conversion of androstenedione and testosterone. Small amounts of progesterone are made by the adrenal gland. After

menopause, androgens do not change uniformly. Androstenedione, the principal ovarian androgen, is reduced by about 50%. The adrenal gland contributes 80% of the postmenopausal androstenedione, while the ovary continues to produce 20% (Chang & Judd, 1981).

Monitoring endogenous production so that exogenous dosages can be individually prescribed would appear to contribute valuable information to the female and her physician. However, existing tests are expensive and can often be unreliable. Most postmenopausal hormone therapy is intended to increase the blood levels of estradiol. The hormone progesterone is not normally produced at all in postmenopausal women. When progesterone is given to menopausal women (mainly for women with an intact uterus) it is primarily used to protect the endometrium (Bass, Westhoff, & Bush, 1995).

Currently oral forms of estrogen and progesterone, Premarin® and Provera® in the form of tablets, are the most widely used exogenous female sex hormones. With Premarin® the standard human dose for estrogen replacement therapy is 0.625 mg daily. It protects against postmenopausal osteoporosis (Type I) and cardiovascular disease in the majority of postmenopausal women (Stanczyk, et. al, 1995). The annual rate of endometrial hyperplasia (excessive proliferation of normal cells) at this dose is estimated to be between 7% and 15%. With Provera®, the most widely prescribed human progestational agent for use in conjunction with estrogen replacement therapy dosages vary from 2.5 mg, 5 mg and 10 mg. The regimen that is most convincingly shown to prevent hyperplasia has been 10 mg at days 13-28 of each cycle. This above described treatment is most

generally prescribed by the physician following either surgical or natural menopause (Grady & Cummings, 1995).

While these exogenous female sex hormones have positive cardioprotective effects, they have also been reported to increase macronutrient food intake and thus increase caloric intake (Wade and Schneider, 1992). More recently it has been reported that the addition of 10 to 15 lbs. of body weight gain has been shown to occur when circulating estrogen levels begin to decline in the perimenopausal years (Wing, 1992; Troisi, et al, 1995). Physiologically, perimenopause is a time of erratic menstrual periods prior to menopause between the ages of 35 and 45. Estrogens have numerous actions on several biologic systems. Such systems include lipid metabolism, coagulation parameters, and blood pressure.

The effect of hormone replacement therapy on carbohydrate metabolism in menopausal women has been reviewed by Notelovitz and Tonnessen (1993). Early markers of negative effects were short term with no effects on body weight increase. Estrogen treatment was shown to have a bi-phasic effect; abnormal glucose tolerance with a normal insulin level commonly found within 3 months of treatment, followed by normalization of the glycemia when treatment is extended beyond one year. Progestogens have adverse short-term effects on carbohydrate metabolism and on lipid and lipoprotein profile, lowering the ratio of high-density lipoprotein: low-density lipoprotein (HDL:LDL) cholesterol compared to unopposed estrogen replacement therapy (ERT) (Gambrell, 1995).

Longer term trials of more than I year, including the recently published PEPI trial (The PEPI Writing Group, 1995), indicate that time modifies these

effects. The duration of treatment was concluded to be important. This was first commented upon by diPaola, Robinson and Nicholson (1970), who noted that with estrogen treatment most of the abnormal glucose tolerance tests occurred between the first and third months of treatment and decreased thereafter until the ninth month, by which time normal blood glucose levels had returned. Therefore those who experience early appetite shifts may find that with time normalization occurs.

In extreme cases the profile of endogenous female sex hormones can be monitored, however in the population dosages are generally based on symptomology provided through patient communication with her physician. Plasma levels of FSH, LH and estradiol were measured serially in eleven premenopausal patients before and after hysterectomy. One week after the operation an incremental dosage regime of conjugated estrogens in tablet form was commenced on the basis of two weeks of therapy with each dose interspersed with two weeks without therapy. FSH, LH and estradiol levels were measured at the end of each period with and without therapy. Estradiol levels fell within 24 hours of operation while FSH and LH levels rose gradually. In only one instance did conjugated estrogens succeed in reducing FSH to premenopausal levels, and that was at a dosage of 2.5 mg. It should therefore be noted that usual conjugated estrogen treatment after surgical menopause does not represent physiologic "hormone replacement therapy," if defined as the dosage to maintain premenopausal circulating concentrations of reproductive hormones (Utian,1978; Lerner, 1995).

Additional physiological studies in postmenopausal women are needed. An early study by Notelovitz (1982) was conducted to examine blood

pressure and body weight of postmenopausal women after one year of estrogen-progestin therapy. Twenty naturally menopausal women received either 0.625 or 1.25 mg conjugated estrogens with 10 mg medroxyprogesterone acetate for one year. Eighteen women of similar age and menopausal status were followed for the same time period with no treatment. Blood pressure and body weight were assessed at baseline, six months, and one year. No significant changes in blood pressure or body weight occurred in either group nor were there any treatment-related differences between the groups. More recent data (The PEPI Writing Group, 1995) indicate preliminarily no significant differences in body weight gain in postmenopausal females taking hormone replacement therapy (n=875) over a three year study. This data is expected to be reported in detail in later publications.

Few studies have examined in-depth the implications of female sex hormones on food intake and body weight. Many of the earlier studies were conducted on normally cycling younger females. One such recent study examined the changes in dietary intake, urinary nitrogen and urinary volume across the menstrual cycle in women confined to a metabolic unit. These subjects were maintained at a constant activity level, and fed an ad libitum, rotating, staff-weighed diet. No significant changes in intakes of energy, protein, and fat occurred throughout the menstrual cycle (Fong & Kretsch, 1986).

One of the few current reports on postmenopausal women utilized a written survey to examine body weight gain in menopause (Wurtman, O'Leary, & McDermott, 1992). This survey of free living subjects (n=490) asked for

reports on changes in body weight, food intake and activity levels in the early postmenopause. The majority reported weight gain since the onset of menopause, regardless of whether menopause was natural or surgical, and whether the subjects were obese or nonobese prior to menopause. This report revealed that weight gain occurred among 64% of the women who were normal weight prior to menopause and among 96% of the women already obese prior to menopause. The traditional perceived causes of weight gain: increased caloric intake, decreased activity, did not seem related to weight gain in the women involved in the study. Women who were not obese prior to menopause gained an average of 10-15 lbs; women who were obese gained an average of 21-23 lbs. The National Health and Nutrition Examination Survey (NHANES III) study does not currently report body weight parameters in this segment of the population. A $625 million study entitled "The Women's Health Initiative" is presently being conducted charting body weight changes over a 15 year period. These results should provide much of the missing information (Healy, 1993). The measurement of fat intake and body weight is included in the experimental design. Reports should be available in 2010.

A research study on body weight increase in 290 women was recently conducted (Wing, 1992). The study reported that age, and not menopausal status, was shown to be the cause of body weight increase. Other literature confirms that the hormonal changes in menopause, are associated with changes in body composition and fat distribution. With time, the postmenopausal female decreases in lean body mass and increases in fat mass (Young, et. al., 1963). Ley and colleagues (1992) investigated the differences in body composition and regional fat distribution among men and

pre-and postmenopausal women. Using dual-energy X-ray absorptiometry (DEXA), they found that postmenopausal women had 20% greater fat mass than premenopausal women. An increase in the percentile of abdominal fat during early menopause also was reported (Haarbo, et al., 1991). However, the latest information (Troisi, et al., 1995) shows a change in distribution to be more pronounced than percentage of body fat changes, and that waist to hip ratios were significantly lower in postmenopausal women taking exogenous female sex hormones.

The National Research Council Committee on Diet and Health: Implications for Reducing Chronic Disease Risk, reports that these shifts in body fat distribution in extreme degrees of obesity increase the risk of coronary heart disease. Shifts in upper body fat has been shown to be an indicator of the predisposition to cardiovascular disease and diabetes in the female. A more important fact which involves a greater percent of the mature population are the modest degrees of overweight, and the implications on risk assessment. The issue of moderate body weight gain has been distorted by the popular press and has been used synonomously with obesity. It should be noted that moderate body weight gain has also been associated with increased immunity and longevity (Bouchard, 1995).

HORMONAL IMPLICATIONS ON HEALTH AND DISEASE

The issue of body weight, fat intake, longevity and subsequent implications on morbidity as reported in the current media has caused national concern. Dr. Richard Atkinson, president of the American Society for Clinical Nutrition stated in a recent report that $33 billion dollars were spent on weight loss in this country last year. Research surrounding the segment of the

population incurring these expenses should be conducted. This manipulation of the public in the U.S. is not seen globally. A French study showing higher national levels of fat consumption but lower incidences of heart disease is one example (Criqui & Ringel, 1995). The mission statement of the newly formed Office of Research on Women's Health shows that research surrounding prevention, diagnosis, and treatment of illness and disease conditions in women should be conducted. These studies may reveal a very different profile than is currently perceived in this country.

Current reports correlate increased fat intake and implied increased body fat stores with disease states. These may not be confirmed in future research. Age and genetics may be the primary reasons for body weight increase. The transition from premenopausal to postmenopausal status has been related to an increase in coronary heart disease risk. An additional fact often not mentioned in mortality studies is that "reason for death" on coroner's reports in the past only included 4 categories, and heart disease was listed first. Mortality records in this country may reflect skewed bias in favor of heart disease for this reason. The effect of weight changes on coronary disease risk factors in a population-based sample of 485 middle-aged women spanning three years (Wing, et al., 1991) revealed that weight gain was significantly associated with increases in blood pressure and levels of total cholesterol, low-density lipoprotein cholesterol, triglycerides and fasting insulin. However, it should be noted that these increases were associated primarily with age. Aging and menopause are natural phenomena which should be considered and correlated statistically as these results are reported. These physiological parameters are not surprising.

The correlation between weight change and coronary heart disease (CHD) in women was recently presented (Willett, et al., 1995). The researchers assessed the validity of the 1990 U.S. weight guidelines for women. The results show that in a follow-up of the Nurses Study (total of 115,818 women 30 to 55 years of age without a history of coronary heart disease), that only (1,292) 12% cases of coronary heart disease were ascertained. These data which were analyzed after controlling for age, smoking, menopausal status, postmenopausal hormone use, and parental history of CHD, revealed that the women who gained weight within the BMI range of 18 to 25 kg/m2 from 18 years of age were strongly predicted to develop CHD. Willett, et al. report that higher levels of body weight within the "normal" range, as well as modest weight gains after 18 years of age, appear to increase risks of CHD in middle-aged women. These data are currently in question. National averages show that only 12% of the population is presently affected, and that baseline data calculated from body weight at the age at 18 may not reflect meaningful information.

Further, Willett et al. reported that current recommended weight guidelines may be falsely reassuring to that segment of the population of women older than 35 years who fall within those guidelines. The report states that some risks of CHD could be avoided if body weight guidelines were lowered. This report may cause unwarranted alarm, and create the mental stimulation for the astronomical $33 billion spent on weight loss as mentioned earlier by Dr. Atkinson. Further increases in anorexia nervosa and bulimia may be seen as the result of the information reported by Willett, et al. In all fairness, it should also be noted and reported that 88% of the women did not

suffer from the reported cardiovascular weight related implications. The pattern of alarming 100% of the population when 10-15% suffer from a health-related problem has become the norm in this country and is often used to market high cost food items. This form of marketing can be termed a manipulation of the health conscious aging American and has become a major source of income for vendors. The scientific accuracy upon which these claims are made should be questioned and examined further.

The Center for Science for the Public Interest may be the source of the current perceptions and misconceptions on fat which are being manipulated and used for marketing ploys in the U.S. population. Recently a report revealed the self-selected contents of the refrigerator at the Center as being high in fat for its employees. Clearly the body fat issue needs definitive and conclusive evidence not currently in place in the literature as it relates to health parameters and disease states. These human eating behavior parameters are the focus of this research.

The question of hormonal effects on food choice and future impact on health parameters is not known. Female sex hormones are an obvious choice to examine since increasing numbers in the female population are choosing a supplemental form to address health related implications. The most recent data suggest that estrogen therapy reduces heart disease (The PEPI Writing Group, 1995). These reports show that exogenous estrogen suppresses hepatic lipase activity, thereby raising the level of high-density lipoprotein cholesterol (HDL). Barrett-Conner and Bush (1991) noted that between 25% and 50% of the beneficial action of estrogen on coronary heart disease risk could be ascribed to changes in HDL-cholesterol and LDL-cholesterol.

Hormone replacement therapy (HRT) is currently accepted for relief of menopausal symptoms, and for the reduction in risk for osteoporosis and cardiovascular disease. With increasing numbers of females choosing HRT, long term unknown health concerns should be addressed. One fact seems very clear: preliminary clinical and laboratory work-up prior to hormone replacement therapy in postmenopausal women (Pardo, et. al., 1992) should consist of mammography test for malignancy exclusion, confirmation of menopause by FSH and estrogen, and also the confirmation of normal lipid metabolism. Baseline data is essential to the strength of reported results.

Additionally, the assessment of dosages may be an area of concern for postmenopausal women considering hormone replacement therapy. It should be noted that it is rare that investigators have included biologic measures of serum female sex hormones before, during or after drug administration. Without such data, dosages administered and the classification of pre-, peri-, or postmenopausal status provide only a rough yardstick with which to evaluate subject or treatment characteristics. One such study revealed the effects of estrogen-progestin replacement therapy in postmenopausal women (Ortega, Cuadros, Gonzalez, & Ruiz, 1993). Serum levels of LH, FSH, estradiol and progesterone were measured. Measurements were collected before and after four different estrogen-progestin replacement therapies. The results showed that serum levels of LH and FSH dropped significantly in all four groups, that estradiol levels increased in all groups but that progesterone levels remained relatively unchanged.

New studies on micronized progestins (the natural form found in such food sources as soybeans and yams) as opposed to medroxyprogesterone

are providing evidence of decreased side effects (which affect compliance) with micronized progestins (The PEPI Writing Group, 1995). Negative side effects can be premenstrual syndrome, bloating, depression, weight gain and uncontrolled eating bouts. The micronized progesterone used in the PEPI trial was produced by Schering Plough, Kenilworth, NJ. Synthetic progesterone produces different more pronounced side effects than the natural plant sources (Hargrove, et al., 1989). However, micronized progestin bioavailability may be lower and dosages may therefore need to be increased. Progesterone receptor site levels are significantly lower in postmenopausal women (Becker, et al., 1990). Side effects may directly correlate to compliance rates in the postmenopausal female. Additional research showing endogenous levels prior to administration of dosages and comparisons with medroxyprogesterone (Provera®) are needed in the human postmenopausal female. More specifically, mechanisms and physiological implications of all sources of exogenous female sex hormones should be investigated.

The varieties in the mode of presentation of exogenous sex hormones has increased recently: oral, suppositories, vaginal creams, patches and subdermal implants given cyclically, continuously or at 3 to 6 month intervals. The dosages that provide the greatest degree of cardioprotection with minimum side effects should be investigated. An earlier study of postmenopausal women given exogenous esterified sources of estrogen 0.625 mg (Estratab), esterified estrogen 0.625 mg plus 1.25 mg methyltestosterone (Estratest H.S.), or conjugated estrogen 0.625 mg (Premarin®), showed no significant differences among treatment groups (Jurgens, et. al.,1992). Serum levels of estradiol remained unchanged. There

are often correlations between estrogen receptors and estradiol in postmenopausal women which provide biomarkers for cancer and cardiovascular disease (Perez-Lopez, et al., 1993). The demand is increasing as cardioprotection studies are released and osteoporosis prevention is further investigated. In the absence of serum tests for steroid levels, the current method of measurement requires verbal feedback from the patient to the physician. And very often hormone replacement is discontinued before the physician is contacted.

Much of the current popular interest in exogenous female sex hormones comes from the effect on a disease state such as CHD or osteoporosis. Although the initial decision often surrounds compensating for negative menopausal symptoms, the continuation of exogenous female sex hormones may have other less obvious reasons and compliance is abandoned. The implications of body weight gain as affected by varying dosages of HRT in the healthy female who chooses hormone therapy have not been thoroughly investigated. Higher levels of progesterone often prescribed for women with a family history of breast cancer may initiate a fat appetite which can further compound the problem (Rolls personal communication, 1993, (Ob/Gyn. physicians personal communications, 1994).

A recent study conducted at the University of Vienna, Department of Obstetrics and Gynecology, shows that after long term therapy with estrogen implants, a 13% increase of prolactin was reported (Metka, et al., 1994). This increase seems to be dose related. Further work is needed to examine body weight implications. Another related study by Bennet and Ingram (1990) was designed to investigate the possible mechanisms by which dietary fat may

influence the development of breast cancer by influencing the concentration of female sex hormones. They investigated the effect of the type of fat consumed on concentrations of female sex hormones in serum. They found that when nutrient consumption was correlated with hormone concentrations, prolactin was directly associated with fat consumption, and sex-hormone-binding globulin was inversely associated with fat consumption (particularly cholesterol consumption), and that the proportion of nonprotein-bound estradiol was directly associated with complex carbohydrate consumption.

Currently, a laboratory in Boston is reducing prolactin levels and altering body weight without altering caloric intake. Cincotta and Meier (1986) have shown that altering circadian rhythms can create desirable metabolic outcomes on body weight and diabetic states. Reductions of prolactin secretion by bromocriptine treatment for 24 days reduces fat stores in hamsters by 25-49% compared with control animals. However, body weight and food consumption were not affected. These results document the important role for prolactin in regulation of fat metabolism and indicate that bromocriptine might be used to decrease fat stores. More recently, a report from this laboratory has shown a 600% decrease in caloric intake in the OB/OB mouse with a return to normal body weight following a state of obesity (Dr. A.H. Meier, personal communication, 1995). Their work takes us beyond the current findings in the female rat and may in the future provide answers to the perplexing questions surrounding body weight gain and various disease states such as diabetes mellitus.

There appears to be a growing body of evidence that hormonal profiles affect food intake choices. The clinical analysis of hormonal levels prior to

dosage determination and prescription in the human may be appropriate in the future for a variety of reasons.

The management of the peri- or postmenopausal woman, whether symptomatic or asymptomatic, involves a careful assessment of the problems and expectations. The effects of the physiological implications of menopause and treatment on immediate and long-term health must be taken into account. This may involve aspects of medical topics as diverse as physiology, gynecology, endocrinology, nutrition, psychology, oncology and cardiology. Although the benefits of estrogen therapy are well established, the response to therapy must be carefully monitored, seeking out adverse effects both in individuals and populations.

HORMONAL EFFECTS ON ENERGY BALANCE

Although very little information is available from the human studies, many scientists have measured the effects of both endogenous and exogenous fluctuating levels of gonadal hormones in animal models. The most descriptive work was reported by Wade and Gray in the 1970s. Much of the earlier research to measure control of body weight (e.g. food intake and voluntary exercise) was conducted in the albino rat (Wade & Zucker, 1969; Wade & Gray, 1979; Wade, Gray, & Bartness, 1985; Wade and Blaustein, 1978). In female rats estrogens decrease food intake, body weight, and adiposity, while progestins increase these parameters (Wade and Schneider, 1992). In the male rat adiposity is suppressed by testosterone in a wide range of diseases. In female rat studies, the age of the rat is often not reported. Due to reproductive four day estrus cycles and gestational patterns, this information appears relevant to progesterone levels and research results.

While Wade's research in the animal has provided a great deal of information on the basic endocrinology of body weight as affected by female sex hormones, very little is known about the physiological or biochemical mechanisms by which ovarian and testicular hormones act to influence body weight, total body composition and behavioral food choices in the human. In a theoretical review Wade and Gray (1979) reported selected aspects of sex hormone actions on body weight and composition, metabolism, and behaviors in the rat. This work is needed in the human to show empirically the investigations conducted in the rat, female sex hormone effects on adiposity and the relationship to eating behavior and food choices. If the results of earlier work in the animal can be applied to the human, then gonadal steroids may be shown to influence adiposity by direct action on a few peripheral adipose tissues which control triglyceride storage with subsequent metabolic implications. If this is true, changes in macronutrient food choices and caloric regulation may be altered by varying levels of circulating hormones with resulting metabolic benefits over time (Jorgensen, et. al., 1994).

Behavioral and physiological controls of macronutrient choices and energy balance vary with reproductive condition in female rats. Experimental manipulations of circulating hormone levels (ovariectomy and hormone replacement therapy) indicate that the regulatory changes that are seen over estrus cycles are due in large part to fluctuations in levels of estradiol and progesterone (Wade, 1976; Wade & Gray, 1979). Estradiol acts both centrally and peripherally to induce a coordinated array of changes in the procurement, ingestion, metabolism, storage, and expenditure of metabolic fuels. In young

rats, estradiol decreases the intake and increases the expenditure of energy, thereby decreasing body fat stores.

In general, progesterone reverses nearly all of the effects of estradiol and, thus promotes fat storage in rats. In the younger model, it has been suggested that body weight changes are somehow related to estradiol-induced shifts in a hypothetical body weight setpoint. That is, changes in voluntary exercise and metabolic expenditure persist only until body weight is brought into line with a new regulated level. A recent release in the New England Journal of Medicine conducted by scientists at Rockefeller University reported dynamic effects of setpoint in both obese and non-obese subjects (Leibel, Rosenbaum, & Hirsch, 1995). Earlier reports by Fantino and Cabanac (1984) have shown hoarding behavior as an indicator of food deprivation effects on set point in body weight studies.

In adult female rats, withdrawal of ovarian hormones by ovariectomy induces a hyperphagia (hunger) and weight gain which lasts for approximately one month (Wade & Gray, 1979). Then the hyperphagia subsides, and body weight is maintained at 20-25% above the level of sham-operated controls (Gentry & Wade, 1976; Salhanick, et al., 1969). Running wheel activity is permanently suppressed by ovariectomy (Wang, 1923). The effects of the ovariectomy can be reversed by treatment with estradiol alone. Treatment of ovariectomized rats with physiological doses of estradiol produces a transient hypophasia (satiety) and decline in body weight. Body weights of estradiol treated rats are lower than, as well as parallel to, the ovariectomized controls (Mook, et al., 1972; Wade, 1975). As long as estradiol is given, running wheel activity remains elevated (Wade, 1975). Leshner and Collier (1973) found that

ovariectomy doubled carcass fat content in weanling female rats. Treatment with estradiol has been shown to reverse the increase in adiposity caused by the ovariectomy (Roy & Wade, 1977; Salans, et al, 1971).

Earlier studies have shown that progesterone has no effect on food intake, voluntary exercise, body weight, or carcass composition in ovariectomized rats (Galletti & Klopper, 1964; Hervey & Hervey, 1967; Rodier, 1971; Ross & Zucker, 1974). However, in the presence of estradiol (in cycling females or in ovariectomized female rats given estradiol), treatment with high doses of progesterone (5 mg/day) increased body weight and food intake. The hyperphagia and increased body weight gain during progesterone treatment are transient, just as after the ovariectomy (Hervey & Hervey, 1967; Roberts, et al., 1972). During pregnancy and pseudopregnancy, when plasma progesterone levels are elevated, eating (Hashimote, Hendricks, Anderson, & Melampy, 1968), weight gain, and fat depositions are also elevated (Knopp, Saudek, Arkey, & O'Sullivan, 1973; Slonaker, 1924; Wade & Zucker, 1969), as they are during exogenous progesterone treatment.

Carcass analyses indicate that the weight gain during progesterone treatment in the presence of estradiol is due primarily to the increase of fat tissue (Galletti & Klopper, 1964; Hervey & Hervey, 1967), similar to that seen with ovariectomy. It should be noted that the age of these animals is not in evidence in all cases, and that no studies are reported in the postbreeder rat.

Sexual and hormonal variables have been shown to significantly affect the rat's preference for sweet taste. Taste tests in rats have been conducted in water bottle fluid consumption flavored with quinine (bitter) and sweet (saccharin). The decrease in fluid consumption was proportionately greater

for intact than ovariectomized females when the water supply was adulterated with quinine sulfate. Quinine acceptance by ovariectomized rats was unaffected by treatment with estradiol benzoate or progesterone alone. In combination, these hormones were effective in decreasing intake of the bitter solution. In intact females, estrogens and progestins appear to act synergistically to increase reactivity to the aversive taste stimulus. The decrease in fluid consumption attributable to the quinine, perhaps is due to the decrease in estradiol and increase in progesterone secretion in pregnant and pseudopregnant females compared to unmated females. It is further suggested that the decrease in sweet effect is due to the decrease in estradiol and increase in progesterone secretion and subsequent bioactivity characteristics of these endocrine states. Possible neural mechanisms account for these different effects (Wade & Schneider, 1992).

Behavioral change has been widely assumed to be the contributing factor in the changes in body weight and fat depositions which follow fluctuations in ovarian hormones. The action of ovarian hormones in the brain which could alter food intake and voluntary exercise might then influence fat deposition and body weight gain. In the normally cycling rat, there is an excellent correlation between changes in body weight, food choice and level of activity. During estrus cycles (when estrogen levels are high) the decrease in body weight occurs as a result of increased voluntary exercise and a decrease in food intake (Brobeck, Wheatland, & Strominger, 1947).

However, there are several lines of evidence that suggest that no simple causal relationship exists between sex hormones, behavior, and fat deposition. Fluctuations in food intake and hormonal levels correspond quite

closely but running wheel activity is permanent as long as the exogenous hormonal levels remain constant. The changes in activity persist long after the changes in body weight gain have subsided (Mook, et al., 1972).

Hormone induced changes on body weight are not always accompanied by changes in food intake. Hervey and Hervey (1967) found that progesterone treatment significantly increased body weight in cycling female rats even when the normally-occurring excess food intake patterns were prevented by restricting their food intake to pre-treatment levels. In fact, Roy and Wade (1977) found the ovariectomized females had to be restricted to 80% of their normal consumption to prevent the castrated weight gain. Thus excess intake of calories is not necessary for either progesterone or ovariectomy induced body weight gain and leading to obesity.

A number of studies, mainly conducted in genetically obese and overfed rodents (Rothwell & Stock, 1979; Trayhurn, 1984) have shown that brown adipose tissue may play an important role in the regulation of energy balance. Edens and Wade (1983) also have suggested that brown adipose tissue might be involved in the regulation of energy balance in rats treated with ovarian hormones. A reduction in brown fat thermogenesis is usually accompanied by a reduction in energy expenditure, which in turn leads to a positive energy balance. This hypothesis needs to be examined more carefully before it is assumed to be true in the aging female rat model.

These past studies do not provide evidence of human correlations and have not been continued. The correlation to human health parameters needs to be addressed in future research.

HORMONAL EFFECTS ON FOOD INTAKE IN THE HUMAN FEMALE

Hormonal effects on food intake have not been extensively investigated in the human female. Currently the only macronutirent body weight data are reported in the younger cycling female model during reproductive years. The effects of exogenous female sex hormones in the postmenopausal woman taking hormone replacement therapy have not been investigated and reported. It should be noted that endogenous and exogenous female sex hormones may not be valid comparisons. Chemically, these are not the same, the natural rhythm of secretion release, and the binding of endogenous hormones has not been mimicked by exogenous treatment. The comparison merely provides a point of departure for further investigations in the postmenopausal female taking hormone replacement therapy. It is likely that the two comparisons may not reflect parallel applicable information. With that disclaimer the following information will be examined.

Systematic shifts in energy intake, corresponding to different phases of the menstrual cycle, have been described (Barr, Janelle, & Prior, 1995; Lissner, Stevens, & Levitsky, 1988; Manocha, Choudhuri, & Tandon, 1986; Oram, 1987; Dalvit-McPhillips, 1981, 1983; Abraham, Beaumont, Argall, & Haywood, 1981; Gong, Garrel, & Calloway, 1989; Gallant, et. al., 1987; Pliner & Fleming, 1983). Several studies have shown that in the younger cycling female the menstrual cycle affects caloric intake and energy expenditure, however as earlier stated, no such research studies are found in the literature in postmenopausal women with intake of exogenous female sex hormones. One such well controlled trial of college age females shows that women

consumed about 500 kcal more per day for the 10 days following ovulation (luteal phase) than the 10 days preceding ovulation (follicular phase) (Dalvit-McPhillips, 1981). This was supported in later work by Dalvit-McPhillips (1983) and Manocha et al. (1986); both observed elevated energy consumption during the 10 days before menstruation (luteal phase) when compared with the 10 days after the onset of menstruation (follicular phase). However, Abraham et al. (1981) found that energy intake was at its minimum level around the onset of menstruation (late luteal phase). Several authors have recommended additional research investigating women's health by examining in greater detail food selection or nutrient intake (Dalvit-McPhillips, 1983; Tomelleri & Grunewald, 1987). Pliner and Fleming (1983) found that both body weight and reported food intake were significantly higher during the luteal phase than during the follicular phase in menstruating women. They further report that caloric intake from sweetness (sucrose) preferences declined in the luteal phase when (progesterone levels were elevated) with an absence of such a decline in the follicular phase. Some have suggested that a better understanding of the food intake patterns and body weight gain in women may aid in understanding related diseases and illnesses affected by obesity (Bush, 1990).

Smith and Sauder (1969) stated that in collecting their human data, 85% of those who craved chocolates also included themselves in the group that craved sweets. In a report by G.E. Abraham (1984), a premenstrual tension syndrome subgroup reported cravings for sweets, "mainly chocolate." Because many chocolate foods are high in both sugar and fat content, one cannot conclude that women in previous studies were necessarily craving

sweets. They may have also been craving fatty foods or they may have been craving a combination of sweet/fatty foods. It is also possible that some subjects may experience a craving for chocolate flavor interacting with the sugar and/or fat content of the food. Others may experience cravings for chocolate independent of the sugar and fat content. Therefore, the present investigation was designed so that phase III of the study would measure various fat and sugar levels of chocolate in the female rat model.

Caloric intake can be largely determined by the taste or palatability of food, and many highly palatable foods (including most chocolate foods) are high in sugar and fat content (Weiffenbach, 1977; Rodin, 1980). Obese humans find oral stimuli with a higher fat content (Drewnowski, et al., 1985) or a higher sugar content (Rodin, Moskowitz, & Bray, 1976) more palatable than do normal-weight subjects. Increased taste responsivity to sweets and oral responsivity to fats have often been cited as important factors in overeating and in the development and maintenance of obesity.

The ways in which foods may increase willingness to eat or to satisfy the desire for further food is also an issue of great theoretical significance. Products in which palatability has been raised in order to promote consumption may have the potential for causing overnutrition. In addition to the new types of foods, additives are injected into the food supply on a regular basis. Little is known about the effects of these additives on appetite. It could be of great value to have tables showing the energy-satiety ratio of all the common foods to indicate their potential for causing overnutrition (Heaton, 1981). The study of the mechanisms of appetite is another dimension of this

area of research which is beyond the scope of this paper. But it is of profound interest and applicability to this study and future research.

As earlier stated, to extrapolate data shown in the younger female during ovulatory menstrual cycles to the effects of exogenous female sex hormones in the postmenopausal female may not be accurate. However, it does provide a view of the implications of energy intakes in the luteal as opposed to the follicular phase in younger cycling females. Specifically, Barr, Janelle, and Prior (1995) compared energy and macronutrient intakes across the menstrual cycle in 42 regularly cycling vegetarians and nonvegetarians. Temperature records were quantitatively analyzed to determine whether cycles were ovulatory, and if so, the date the luteal phase began. Analysis was based on diet records matched with temperature analysis results. Higher caloric intake was observed during the luteal phase. The contribution made in this data suggests that 30% of the cycles may be anovulatory or have a short luteal phase, and that energy intake of women differ between menstrual cycle phases when the menstrual cycle is normally ovulatory. They recommend that in cross-sectional studies, failure to consider menstrual cycle phase could have important implications, especially if small numbers of subjects are studied. They further recommend that the time of ovulation must therefore be documented because energy intake differs only when cycles are ovulatory. It should be noted that this study may provide evidence of the conflicting reports seen in earlier literature such as Abraham and his colleague's (1981) who reported energy intake at a minimum just before menstruation.

To examine estrogen and progesterone effects separately is not possible in the younger human model. Progesterone levels are elevated during the luteal phase but not to the exclusion of estradiol (Becker, et al., 1990). Animal work provides the strongest body of evidence, which indicates greater caloric intake and body weight gain when progesterone levels alone are elevated (Wade & Schneider, 1992). Evidence from recent reports indicates a greater caloric intake, snack food consumption, and body weight during several premenstrual days compared with the postmenstrual period (Barr, Janelle, & Prior, 1995; Dalvitt-McPhillips, 1981; Dalvitt-McPhillips, 1983; Pliner & Flemming, 1983; Lissner, Stevens, & Levitsky, 1988; Lyons, et al., 1989; Gong, et. al., 1989) in contrast to the follicular phase when estrogen levels are elevated. These studies provide interesting information to those interested in investigating the effects of exogenous female sex hormones, estrogen and progesterone in the postmenopausal female. Hormonal effects on food intake, body weight and the set point surrounding the timing of these biological processes should be investigated further in the human postmenopausal model. New information is appropriate on this growing segment of the population which has previously not been studied.

HORMONAL EFFECTS ON APPETITE, TASTE AND PALATABILITY

Positive hedonic judgment or pleasantness of food taste such as sweetness in chocolate can lead to a preference for consumption and increased intake of a particular food group (Drenowski, et al., 1985). Appetite and tastes may be influenced by both endogenous and exogenous female sex hormones. The timing and sequence of events leading to subsequent food consumption and body weight increase has not been determined (Friedman,

1990). Further, the impact of hormonal effect on food choice to the exclusion of other more nutrient dense foods and subsequent effects on total health in the female should be critically examined.

The question of increased palatability influencing food choice in the face of a deprivation of essential vitamins and minerals necessary to maintain bone density in menopause could provide additional research questions in the aging female. Do eating patterns in aging cause the physiological markers of degeneration with subsequent disease states and high cost health care in the aging segment of the population? If the answer is yes, then (a) what is causing the alterations in appetite and (b) what can be done to improve the health status and correct nutrition related disease implications. Again, that is beyond the scope of this paper but could ultimately affect future research and the preventive medicine and wellness picture as managed health care is implemented. Scientists involved in brain research as it controls systemic responses are providing the most interesting current results.

However, knowledge of the mechanisms which control food intake and body weight has been surprisingly meager. Recent work at the University of North Carolina School of Medicine in this area has shown that the surface of the gastrointestinal tract may be more than the site of absorption of nutrients; it may be a preabsorptive reservoir of signals which would be released by contact with ingested food (Whitehead, personal communication, 1995). These preabsorptive signals would then act to control meal size, regulate length of meal intervals or both. This concept has been strengthened by continuing chemical discoveries of neural and hormonal gastrointestinal peptides which might serve as satiety signals. The fact that many of these

same peptides and their receptors are also present in brain tissue has generated acceptance of the idea that brain stores of certain peptides might exert direct actions of feeding behavior, unrelated to any peripheral effects.

The classic small intestinal hormone cholescystokinin (CCK) when administered systematically can cause a reduction in food intake in rats. When it is released by food ingestion, it functions as a negative feedback signal to limit meal size. This inhibitory action has been observed in many animal species, including sub-human primates, across a wide variety of test conditions (Gibbs, Young, & Smith, 1973). The action is strongly dose-related and is behaviorally specific. Although a large amount of evidence has been established, the fact that exogenous CCK reduces short-term food intake is evident. The mechanisms of endogenous CCK for the normal termination of eating have not been clearly determined but the CCK hypothesis is emerging as an established finding.

The hormone-releasing action of CCK is believed to elicit satiety. This conclusion was drawn from surgical and chemical lesion studies which have demonstrated that afferent, capsaicin-sensitive neurons of the abdominal vagus are required for the satiety action of CCK injected peripherally. The entry into the brain through afferent neurons of the vagus have their first synaptic relay in the hindbrain (Gibbs & Smith, 1982). Brain sites and pathways involved in processing food intake signals is the specific research focus of many scientists investigating ingestive behavior. Clearly, all the answers are not in place. The following is a summary of mechanisms relating to ultimate food choice.

Pancreatic glucagon was the first peptide tested for a possible effect of food intake signals. Intravenous dosages of glucagon small enough to mimic the rise in plasma glucagon normally seen within twenty minutes after the completion of a meal were shown to reduce test meal size by about 20%. Geary and Smith (1982) reported the efficacy of systematically administered glucagon in producing satiety in a wide variety of animal and avian species. Like exogenous CCK, exogenous glucagon is strongly dose-related, rapid in onset, and transient. Unlike CCK, the effect of glucagon appears quite sensitive to environmental conditions, deprivation states and circadian rhythms. Mildly deprived or nondeprived rats are refractory to the satiety influence of glucagon around the time of dark onset, but quite responsive two or three hours later, or during the mid-light period (Martin & Novin, 1977).

The liver is the major target organ for metabolic actions of glucagon. Novin and his colleagues tested the effects of infusions directly into the hepatic portal vein (Martin, Novin, & VanderWeele, 1978). By this route, glucagon produced a rapid and dose-related reduction in food intake. It appears that afferent fibers of the hepatic vagus are the key element supporting this behavioral effect. These results constitute persuasive evidence that circulating glucagon has a physiological role in controlling food intake.

Bombesin is known to be distributed in mammalian gastrointestinal tract and brain. Intraperitoneal injections of bombesin produced large, dose-related reductions of food intake in rats. Several studies suggested that the inhibition of food intake reflected a true satiety effect of the peptide which was not simply secondary to malaise or generalized discomfort (Murrahainen, Kissileff, & Thornton, 1983). As with CCK, the satiety effect of peripherally-

administered bombesin produced a significant inhibition of food intake at test meals without producing significant side effects.

Insulin, administered peripherally in large doses, has long been known to provoke feeding in animals and elicit reports of hunger (Lovett & Booth, 1970). In 1980 VanderWeele suggested that smaller doses of insulin closer to the physiological range would reduce food intake when delivered chronically to rats (VanderWeele, Pi-Sunyer, & Novin, 1980). Chronic reduction of insulin was shown to be due to a decrease in individual meal size. Woods and Porte (1977) have suggested that insulin may play an important inhibitory role in feeding behavior by acting directly on the brain. This places insulin not in the short-term control of individual meal size, but rather as a long term monitor of the adipose tissue stores of the body. This hypothesis rests on several strong supports: (1) plasma insulin is well known to vary with the fat mass; (2) in turn, insulin levels in cerebrospinal fluid vary after a lag period, with plasma insulin (Woods & Porte, 1977), (3) insulin and insulin receptors can be found within the brain, (4) infusions of small amounts of insulin directly into the cerebrospinal fluid of rats and primates reduces daily food intake and body weight, without raising plasma insulin levels, and (5) although plasma insulin levels are elevated in obesity, these investigators have found that cerebrospinal levels of insulin, brain levels of insulin and brain insulin binding are all low in rodent models of obesity, suggesting that the insulin signal from the adipose tissue mass is not reaching its target sites in the brain (Baskin, Stein, & Ikeda, 1985).

It is of interest that this hypothetical mechanism for the regulation of long-term food intake and body weight appears to interact with at least one of

the putative short-term satiety signals. Although the administration of a very small amount of insulin into the cerebral ventricles failed to affect food intake by itself in rats and in primates, it potentiated a sub-threshold dose of CCK, and the combination inhibited food intake (Woods & Gibbs ,1989).

Somatostatin, found in the gastrointestinal tract and brain appears to act as an inhibitor of multiple pancreatic and gut functions. Administered intraperitoneally to rats and baboons, it produced a dose-dependent and behaviorally specific inhibition of food intake (Lotter, Krinsky, & McKay, 1981).

Oxytocin, which is synthesized in cell bodies of intra- and extrahypothalamic neurons, also has binding sites widely distributed in the brain tissue. Doses required to reduce feeding after peripheral administration were massive; intracerebroventricular administration of 10 µg/rat produced a marked suppression of food intake, accompanied by increased grooming. This effect was totally reversed by an oxytocin antagonist (Olson, et. al, 1989).

Calcitonin, which is released postprandially into the circulation, reduced food intake in rats and rhesus monkeys following subcutaneous administration and in rats after intracerebroventricular administration. Maximal inhibition occurred several hours following peripheral injection in rats, suggesting that the peptide is not directly involved in the regulation of meal size (Krahn, et. al, 1986).

Corticotropin-releasing factor, a 41-residue peptide which stimulates pituitary ACTH and β endorphin release, also reduce food intake after injection into cerebral ventricles and the paraventricular nucleus of the hypothalamus (Krahn, Gosnell, & Levine,1984).

Circulating pancreatic polypeptide levels rise dramatically at meals, and this peptide has been shown to reduce food intake and body weight after peripheral administration in lean and obese mice (Malaisse-Legae, Carpentier, & Patel, 1977). It failed to reduce food intake in rats (Gibbs, Gray, & Martin, 1980) and has not been tested in other species.

Secretin and gastrin, two classical gastrointestinal peptide hormones, had no effect on food intake in rats, even when large doses were employed (Gibbs, Young ,& Smith, 1973).

Progress in uncovering the physiological mechanisms of food intake and body weight over the years is evident. The major element in this progress is the discovery of the key role of a small group of peptides which link the gastrointestinal tract and the brain. Enterostatin and Neuropeptide Y are two of the newest peptides reported to impact neural mechanisms of appetite and food choice (Bray, et al, 1990). Leptin (Halass, et al., 1995), is the most recent discovery and holds much promise in genetic implications of obesity research. The review of the entire scope of all the current investigations is beyond the scope of this paper.

In summary, certain appetite mechanism patterns seem to be emerging. It appears that several peptides have their initial action peripherally. Second, this initial action is relayed centrally by afferent nerves. A major role is played by the abdominal vagus. Third, the dorsal hindbrain is required for the full expression of meal cessation.

The recent development of highly potent and highly specific receptor antagonists and antibodies for several of these peptides has provided an indispensable tool for assessing the physiological meaning of effects on food

intake produced by the agonists. The mechanisms of food intake and the safety of these biologically potent agents when given chronically are unknown and must be established (Bray et. al, 1990). The uncertainty of the health status of the female taking exogenous female sex hormones (which were not utilized exogenously for centuries) leaves many questions to be answered. However, it behooves the scientific community to address these relevant questions when such a large segment of the population is potentially affected.

The mechanisms reviewed and the way in which the mechanisms influence food choice and metabolic implications have currently not been correlated to the effects of endogenous female sex hormones. Hormonal effects on appetite, taste, and palatability in the human and the correlation to an animal model should be evaluated. To date, the most reliable systemic specific macronutrient food intake research has been conducted in animal models. The following is a report of current and past work which may ultimately lead to the answers affecting knowledge surrounding hormonal influences on food intake and body weight. Reports from both animal and human studies will be included to provide as much information as is currently available in a concise form.

When receiving exogenous estrogen, the female rat shows an increased sugar preference and a decreased fat preference. Also, sugar preference increases with increasing levels of estradiol. Geiselman, Martin, VanderWeele and Novin (1981) showed that when rats were behaviorally responsive to their elevated endogenous estradiol levels they not only showed a significant increase in sugar intake, but they also showed a significant decrease in fat consumption.

In a similar human study, Aaron (1975) reported greater pleasantness of the sweet taste in the follicular phase which lowered during the luteal phase when progesterone levels were higher and estradiol and estrone were lower. In an additional study across the menstrual cycle, Bowen and Grunberg (1990) reported increased sweet food consumption with higher preference ratings during the luteal phase, when progesterone levels are generally elevated. However, it should be noted that their "sweet" foods included combinations of fat and sweet such as chocolate, coffee cake, and gum drops analyzed together. Pliner and Fleming (1983) found that negative alliesthesia i.e., a decrease in sucrose preference typically reported from before to after a glucose load, occurs during the luteal phase but not the follicular phase. The importance of studying not only sugar preference but also fat preference in a controlled female rat model is further suggested by data showing that when endogenous estrogen levels are lower and progesterone is elevated, rats chose significantly more dietary fat (Geiselman, et al., 1981). There are only a few anecdotal reports in the human literature of changes in fat preference across the menstrual cycle, with no evidence in the aging rat or postmenopausal female. It is important to understand the hedonics of specific appetites as affected by female sex hormones.

It is therefore important in this study to systematically examine both sugar and fat content in food choices as seen in separate food cups containing nutritionally balanced sources of nutrients. This study was designed to specifically identify and quantify the effects of exogenous female sex hormones (estrogen, estrogen/progesterone, and progesterone) on macronutrient preferences, sweets, fats and chocolate in the ovariectomized

retired breeder female rat to provide information and preliminary data for future human studies. Based on the physiological parameters currently being investigated in this specific study, the female Sprague-Dawley rat seems to be an appropriate animal model.

The animal literature primarily reflecting data collected in the Sprague-Dawley rat strongly implicates female sex hormones in the control of appetite, food intake, and body weight. When female rats are ovariectomized, food intake and body weight both increase (Schemmel, et al, 1982). However, administration of small doses of estradiol reverses the body weight gain and returns food intake to normal (Wade, et al, 1985). A significant decrease of food intake when estrogen is high with a significant increase in food consumption when estrogen is low was seen in the cyclic secretion of estrogen during the estrus cycle of the rat (Tarttelin and Gorski, 1971).

Later work by Tarttelin and Gorski (1973) with ovariectomized rats showed an increase in food intake and body weight which plateaued roughly one month after surgery. Daily injections of 1.5 µg estradiol benzoate initiated at that time significantly reduced both the food intake and body weight. When estrogen treatment is initiated at the time of ovariectomy, the increase in food intake and body weight is prevented. No influence of progesterone injection (either with estradiol benzoate or alone) was detected. These results suggest that estrogen, but not progesterone, is the ovarian hormone active in the regulation of intake parameters and body weight in the female rat (Tarttelin & Gorski, 1973). Fantino and Brinnel (1986) have shown a direct fluctuation of the body weight setpoint with the ovarian cycle. The implications of this work have direct application to the concept of hoarding behavior as a measure of

increased body fat stores and increased body weight in the ovarian cycle of the female rat. Hoarding has been shown to be a direct correlate to increased body rat stores. Proestrus and diestrus measures were taken. The diestrus period is equated to the luteal phase in the female when progesterone levels are elevated. Higher progesterone levels initiated increased hoarding behavior in the mature Sprague-Dawley female rat.

Another study in rats (Mandour, et al., 1977) demonstrated that 17 β estradiol had a marked suppressive effect on the alpha cells of the pancreas and a minimal effect on plasma insulin release as measured in the portal vein. The invasive nature of this study is not appropriate in humans. However, if the data are extrapolated to the clinical situation, it could explain some of the observed biochemical changes seen in women on estrogen therapy, as evidenced by lowered fasting blood glucose levels (because of relative fasting hyperinsulinemia) and elevated triglycerides (Bush, 1990).

Short-term therapy with progestogens (less than 6 months) is not associated with alterations in glucose or insulin metabolism (Spellacy, et al., 1970). However, later work by Notelovitz (1982) shows that time is a strong indicator of effects and that early findings in the first 6 months may change with time. Therefore, the early appetite changes seen when hormone replacement therapy is initiated may not be long term. This fact should be explained to the postmenopausal female by her physician. Such information could affect the decision to begin hormone replacement therapy and the future level of commitment and compliance. However, that information is not currently available. That is a contribution that this scientist hopes to make in the future

in cooperative research with Dr. Notelovitz (Dr. Morris Notelovitz, personal communication, 1995).

Currently the most reliable research in a mammalian model has been conducted in the primate at Bowman Gray School of Medicine (Dr. Thomas Clarkson, personal communication, 1995). Observations of rhesus monkey females indicate a systematic relationship between levels of food intake and endogenous estrogen and progesterone levels. A positive correlation has been noted between the incidence of food rejection and levels of circulating estrogens (Czaja and Roy, 1975). Gilbert and Gillman (1956) showed a sharp decline in primate food intake during the first 6 to 11 days and continued low intake throughout the follicular phase followed by a consistent and maintained rise after ovulation with a peak of caloric intake being recorded between 2 and 7 days before menstruation. Dr. Thomas Clarkson at the Bowman Gray School of Medicine, Department of Comparative Pathology, is currently measuring food intake involving phytoestrogens from soybean and the effects on body weight and cardiovascular disease in the primate. His earlier work provided the basis for the research design for the PEPI trial. Research in appetite, macronutrient intake and body weight with hormonal effects is needed (Clarkson, personal communication, 1994).

EATING BEHAVIOR: ENERGY BALANCE MECHANISMS

Aside from all the uncertainty surrounding mechanisms and biological mysteries, the body is a marvelous organism with built in homeostatic mechanisms. Body weight and food intake are carefully regulated in most laboratory animals, and humans by a variety of physiological and behavioral control mechanisms (Balagura, et al, 1981; Code, 1967; Hervey, 1969;

Morgan, 1965). Although short-term errors do occur in this regulation, long-term regulation is amazingly accurate, and adults of most mammalian species gain weight only very slowly with age (Hervey, 1969).

However, although weight is carefully regulated, it should be noted that body weight setpoint is not immune to physiological and environmental fluctuations. A variety of factors, including diet palatability (Corbit & Stellar, 1964), environmental temperature (Brobeck, et al, 1947), relative levels of various metabolic hormones (Woods and Porte, 1977), and the opportunity to exercise in running wheels (Leshner and Collier, 1973) affect body weight level in rats. Additionally, neurological damage may also raise or lower body weight setpoint (Hoebel & Teitelbaum, 1962; Powley and Morton, 1976). Of interest in this research, and increasingly more relevant are the effects of exogenous female sex hormones on caloric regulation. These female sex hormones should be added to the list of factors affecting behavioral and physiological regulation of body weight.

We have summarized what is known about the effects of ovarian steroids on energy balance and caloric conversion in younger rat models, and earlier human studies. This information and the principles derived from this earlier research have provided the basis to examine this hypothesis in postbreeder female rats. Eating behavior patterns in the ovariectomized postbreeder female rat may provide the model to investigate the effects of exogenous female sex hormones on food intake and body weight in the mature female of other species. Female sex hormones have important effects on behavioral and physiological controls of energy balance in a wide range of mammalian species. Work with laboratory animals reveals that fluctuations in

circulating estradiol and progesterone levels produce coordinated changes in ingestion, metabolism, storage, and expenditure of metabolic fuels. In rats, estradiol can act directly in the brain to alter food intake and voluntary exercise. Subsequent neuroendocrine messages are then translated into actions in the adipose tissue, muscle, and liver to influence metabolism and the storage of metabolic fuels. The effects of estradiol on food intake are due, at least in part, to direct hormone actions in the brain (Wade and Schneider, 1992). Early work using intracerebral hormone implants suggested that estradiol acted in the ventromedial nucleus of the hypothalamus (VMH) to decrease food intake (Beatty, et. al., 1975; Jandowiak & Stern, 1974; Nunez, et al., 1980; Wade & Zucker, 1970). However, more recent work indicates that the paraventricular nucleus (PVN) of the hypothalamus, rather than the VMH, is the principle regulator of estradiol on food intake (Butera & Beikirch, 1989; Czaja, Butera, & MacCaffrey, 1983; King & Cox, 1973; Butera, 1995, personal communication). These data seem to indicate that estradiol can act to decrease food intake in rats, and that it plays an essential role in the effects of estradiol on food intake. Little else is known about the mechanisms of estradiol and other female sex hormones on food intake. The estrogen receptor may hold the answer in future investigations.

Very little is known about the effects of ovarian steroids on energy balance in women, although food intake and metabolism are known to vary with reproductive condition, including menstrual cycles, and menopause. Virtually nothing is known about the macronutrient choices as affected by exogenous female sex hormones on energy balance and subsequent physiological outcomes in postmenopausal women. With recent evidence that

hormone replacement therapy is cardioprotective in the postmenopausal female (The PEPI Writing Group, 1995), information regarding body weight parameters which could affect the rate of compliance is needed. Fluctuations in body weight over a thirty day period represent hormonal influences on setpoint as Fantino and Brinnel (1986) have shown. Short term perceptions of luteal phase or progestin initiated body weight gain may not be related to total body weight over the life span but may be indicative of macronutrient choices with varying caloric conversion rates. The implications may be short term or long term depending on an array of variables yet unmeasured. The investigation seeking these answers will be a continuing dimension to future research related to disease states.

SUMMARY OF THE IMPLICATIONS OF THIS RESEARCH

The number of females in this country taking daily exogenous female sex hormones has increased for the past 30 years and is currently at an all time high (Notelovitz and Tonnessen, 1993; Wallis, 1995). The numbers are increasing daily as postmenopausal baby-boomers choose hormone replacement therapy during menopause. In earlier days of medical practice, hormone replacement therapy was simply called estrogen replacement therapy. When research revealed the predisposition to cancer in those females who were taking "unprotected" estrogen, progesterone was added to the daily or monthly oral intake of exogenous hormones. "Currently, the variety of prescriptions used to treat menopausal symptoms has expanded to include: continuous low levels of both estrogen and progesterone taken simultaneously; and cyclical, whereby estrogen is taken for the first 10 to 14 days of the month and then progesterone is added for the last 14 days. In

addition to an array of patches, implants and injections, oral doses of Premarin® (estrogen) and Provera® (progesterone) are the most widely prescribed forms of hormone replacement therapy as earlier stated. The earlier levels of progesterone varied from 2.5 mg to 10 mg. Higher levels of progesterone have been shown to create premenstrual syndrome-like symptoms which often deter continuance and compliance. The evidence of this potential noncompliance may be initiated by nuisance side effects (such as edema, breast tenderness, memory loss, mood swings, and food cravings) (Gambrell, 1995). The question that must be addressed by the physician and the female patient is: what levels of exogenous female sex hormones are cardioprotective and osteoporosis protective without negative premenstrual tension syndrome-like side effects? The physician needs scientific information based on peer reviewed research to prepare the patient, offer solutions, and help to put potential problems in proper perspective.

The most current research findings involving exogenous female sex hormones in the postmenopausal female do not provide these answers. The results of the first efforts to examine cardiovascular health implications of hormone replacement therapy were revealed at the Fall 1994 meeting of the American Heart Association. This first female clinical trial investigating hormonal effects on cardiovascular disease in the Postmenopausal Estrogen/Progestin Investigation (PEPI), reveals that estrogen only, or in combination with a progestin, improves lipoprotein ratios and lowers fibrinogen levels without detectable effects on insulin and blood pressure. Unopposed estrogen is the optimal regimen for elevation of high density lipoprotein cholesterol (HDL-C), but the study reveals that a high rate of

endometrial hyperplasia restricts the use of estrogen alone by women with a uterus. The design of the PEPI study was changed after 12 months of testing when endometrial biopsies revealed a precancerous state in the uteri of a small percentage of the females taking estrogen only. The research did not focus on the effects of exogenous female sex hormones on caloric intake, body weight and subsequent affect on health status and longevity. The Block Questionnaire (Block, et al, 1986) was used to assess food intake patterns but was only collected three times during the three year study. No direct measures in changes of exercise patterns in the subjects were measured. The further analysis of the existing PEPI data base will provide only a brief indication of metabolic parameters affecting hormone replacement therapy (Dr. Trudy L. Bush, personal communication, 1995).

The current study which seeks to answer some of these questions, is aimed at the interrelationships between exogenous female sex hormones, dietary intake, and specific food preferences (especially as it relates to sweets, fats, and chocolate) in the control of appetite, eating and body weight. The ovariectomized retired breeder female rat is used as a model to provide preliminary data and serve as a guide for future studies measuring the same or similar parameters in postmenopausal women. This study allows identification of dietary and physiologic factors that vary with hormonal levels. It is expected that the factors identified in this project will lead to a better understanding of dietary and physiological implications involved in caloric regulation in the postmenopausal female taking exogenous female sex hormones.

CHAPTER 3: MATERIALS AND METHODS

EXPERIMENTAL CONDITIONS

In order to establish hormone levels in a postmenopausal model, postbreeder female rats which were ovariectomized and then implanted with exogenous female sex hormones, were chosen for this study. Retired breeder Sprague-Dawley female rats (Harlan Sprague Dawley, Madison, Wisconsin) weighing 268.1g ± 3.1g, which had been ovariectomized in Wisconsin before shipment, were housed individually in stainless steel hanging wire cages maintained on a 12:12 lighting schedule (lights on at 0700 hr, off at 1900 hr). A control group of sham surgically treated (ovaries were left intact) rats were observed to document food intake and body weight of animals at this aging status who were not manipulated by exogenous female sex hormones or novel food choices. A control group of ovariectomized rats of the same age reflected the normal body weight and food selections of this model with no hormone implants. (An additional group, sham receiving all food choices was originally included in the research design but a high death rate early in the study eliminated this group.) Three other ovariectomized groups were treated with exogenous female sex hormones which were packed into silastic tubing and implanted subcutaneously (Richard, 1986; Geary, et al, 1994; Butera and Beikirch, 1989; Richard, 1995, personal communication). The source of hormone was chosen to most closely replicate endogenous levels in rats and as a reflection of the bioactivity of exogenous female sex hormones in the human. The rats were randomized so that each treatment group would contain a mean body weight of 268.1g ± 3.1. This experimental design resulted in the following treatment groups:

GROUP A Sham:	Placebo (empty silastic tubing)
GROUP B 0V:	Placebo (empty silastic tubing)
GROUP C OV:	17β-estradiol crystalline (15mg)
GROUP D OV:	17β-estradiol (15 mg) and progesterone (200 mg) crystalline
GROUP E OV:	Progesterone (200 mg) crystalline

ANIMALS

Sprague-Dawley female postbreeder rats which were 10 months old and had given birth to 10 litters of pups were chosen for the study. The rats were ordered four months prior to the study so that a homogenous group of females could be procured. Due to the stress of the two surgeries, the heat, or the age of the animals, five died prior to data collection. The animals were transported to the LSU laboratories by air-conditioned plane and truck.

In summary all materials and methods were designed to answer questions posed in chapter I to test the stated hypotheses. The female rat model was chosen because the controlled laboratory environment allows more accurate measures of body weight and food intake measurements than can be obtained in free living subjects keeping dietary records. The application of animal data to human implications is sometimes questioned (Wade & Schneider, 1992). However, the rat model chosen for this study reflects many of the parameters shown in the literature to directly parallel the human female. The rats were ovariectomized for two reasons: a) to remove endogenous hormone production so that controlled levels could be implanted and documented, and b) to mimic the menopausal state in an animal model.

SURGERY

The ovariectomy or sham surgery was performed by Sprague-Dawley prior to shipment. Sham surgery is typically used in research to induce the stress experienced by the other research animals but ovaries are left intact. Rats were anesthetized with ketamine (100 mg/ml) + xylazine, (20 mg/ml) 3 parts ketamine to I part xylazine at 0.1 ml/100 g BW. The incision was on the upper hind quarter along the back and was secured by metal clamps until recovery. After an adjustment period the rats were weighed and assigned to treatment groups according to body weight. The second surgery to implant the hormones was performed 48 hours after arrival. After an incision was made behind the neck, implants packed with crystalline female sex hormones from Sigma were inserted and the incision was secured with silk thread.

HORMONE IMPLANTS

Silastic tubing made by Dow-Corning was cut into single 15mm lengths for the estradiol and into two, 40 mm lengths to accommodate the progesterone. Silastic capsules were prepared from medical grade silicone tubing (1.5 mm i.d., 3.2 mm o.d.). The crystalline form of the steroids 17 β-estradiol or progesterone (Sigma Laboratories), was packed into the silastic tubing. The ends of the silicone tubing were sealed with a wooden plug followed by Medical Adhesive (Silicone Type A, Dow-Corning, Midland, MI) to prevent dispensation through the cut ends. The tubing was then soaked in a water bath overnight to begin the dispensation process. Thus, released hormone into the animal began immediately upon implantation. The osmotic flow through the walls of the silastic tubing has been shown to provide a reliable method of administering female sex hormones (Richard, 1986).

Each rat received three implants: Sham and OV received a single 15 mm placebo, plus two 40 mm placebo empty tubes. E received a single 15mm estradiol and two empty tubes, E&P received a single 15 mm of estradiol and two 40 mm tubes containing active progesterone. P received a single empty 15 mm and two 40 mm tubing filled with progesterone. Rats were allowed 2 days recovery time from the surgery before food intake data was collected. When the rats were sacrificed, the tubes were extracted from the neck area to confirm that no tube was totally depleted of the hormone source (Richard, personal communication, 1995). Results confirmed that no tube was totally empty.

CONFIRMATION OF HORMONE DOSAGES

Previous research has shown these 15 mg of estradiol and 200 mg. of progesterone in silastic tubing provides blood levels of estradiol within the normal physiological range. Dispensation of the hormones from silastic tubing was confirmed at conclusion of study with radioimmunoassay of estradiol and progesterone in serum and bioactivity was verified by uterine weights (Abraham, 1977; Judd & Korenman, 1982).

WATER AND FOOD CUPS

Tap water was placed in inverted water bottles with stoppers and metal drinking spouts and dispensed ad libitum. Food cups were small glass jars which adequately provided easy access to the three food cups in phase I, four food cups in phase II (three for S) and the eight food cups in phase III (three for S). Food cups were secured with metal bracing to decrease the likelihood of spillage. Phase I food choices are seen in Table 3.1, Phase II, Table 3.2 and Phase III, Table 3.3. Spills were collected in small plastic medicine cups and

TABLE 3.1 PHASE I - TEN (10) DAYS MACRONUTRIENT CHOICES, SEPARATE FOOD CUPS OF PROTEIN, FAT AND CARBOHYDRATES

Group A	Group B	Group C	Group D	Group E
12 Rats	10 Rats	12 Rats	11 Rats	10 Rats
Sham Surgery	Ovariecto-mized Surgery	Ovariecto-mized Surgery	Ovariecto-mized Surgery	Ovariecto-mized Surgery
Control Treatment	Control Treatment	Estradiol Treatment	Estradiol/ Progesterone Treatment	Progesterone Treatment
Protein Food Choice	Protein Food Choice	Protein Food Choice	Protein Food Choice	Protein Food Choice
Fat Food Choice	Fat Food Choice	Fat Food Choice	Fat Food Choice	Fat Food Choice
Carbohydrate Food Choice	Carbohydrate Food Choice	Carbohydrate Food Choice	Carbohydrate Food Choice	Carbohydrate Food Choice

TABLE 3.2 PHASE II - TEN (10) DAYS CARBOHYDRATE ANALYSIS SWEET VS NONSWEET

Group A	Group B	Group C	Group D	Group E
12 Rats	10 Rats	12 Rats	11 Rats	10 Rats
Sham Surgery	Ovariectomized Surgery	Ovariectomized Surgery	Ovariectomized Surgery	Ovariectomized Surgery
Control Treatment	Control Treatment	Estradiol Treatment	Estradiol/ Progesterone Treatment	Progesterone Treatment
Protein Food Choice	Protein Food Choice	Protein Food Choice	Protein Food Choice	Protein Food Choice
Fat Food Choice	Fat Food Choice	Fat Food Choice	Fat Food Choice	Fat Food Choice
Carbohydrate Food Choice	(a) Sweet	(a) Sweet	(a) Sweet	(a) Sweet
	(b) Nonsweet	(b) Nonsweet	(b) Nonsweet	(b) Nonsweet

TABLE 3.3 PHASE III - TEN (10) DAYS SWEET FAT AND CHOCOLATE PROFILE WITH FOUR CALORIC LEVELS OF CHOCOLATE

Group A	Group B	Group C	Group D	Group E
12 Rats	10 Rats	12 Rats	11 Rats	10 Rats
Sham Surgery	Ovariecto-mized Surgery	Ovariecto-mized Surgery	Ovariecto-mized Surgery	Ovariecto-mized Surgery
Control Treatment	Control Treatment	Estadiol (15 mg) Treatment	Estradiol & Progesterone 15mg/200mg Treatment	Progesterone 200 mg Treatment
Protein Food Choice	Protein Food Choice	Protein Food Choice	Protein Food Choice	Protein Food Choice
Fat Food Choice	Fat Food Choice	Fat Food Choice	Fat Food Choice	Fat Food Choice
Carbohydrate Food Choice	CHO Food Choice	CHO Food Choice	CHO Food Choice	CHO Food Choice
	(a) Sweet Food Choice	(a) Sweet Food Choice	(a) Sweet Food Choice	(a) Sweet Food Choice
	(b) Nonsweet Food Choice	(b) Nonsweet Food Choice	(b) Nonsweet Food Choice	(b) Nonsweet Food Choice
Chocolate	(a) Choc HF/HS	(a) Choc HF/HS	(a) Choc HF/HS	(a) Choc HF/HS
	(b) Choc HF/LS	(b) Choc HF/LS	(b) Choc HF/LS	(b) Choc HF/HS
	© Choc LF/HS	© Choc LF/HS	© Choc LF/HS	© Choc LF/HS
	(d) Choc LF/LS	(d) Choc LF/LS	(d) Choc LF/LS	(d) Choc LF/LS

Chocolate:
(a) High Fat/High Sugar
(b) High Fat/Low Sugar
(c) Low Fat/High Sugar
(d) Low Fat/Low Sugar

TABLE 3.4 MACRONUTRIENT SELECTION DIETS

	Protein		SW-CHO		Nonsweet CHO		Fat	
	gm	% kcal	gm	% kcal	gm	% kcal	gm	% kcal
Protein	84.8	89.7	4.9	5.1	4.9	5.1	8.8	5.1
Carbohy-drate	4.8	5.1	84.5	89.7	84.5	89.7	8.7	5.1
Fat	2.1	5.1	2.1	5.1	2.1	5.1	67.4	89.7
Total	91.1	100.0	91.6	100.0	91.6	100.0	84.9	100.0
kcal/gm	3.72		3.77		3.77		6.76	
Ingredient	gm	kcal	gm	kcal	gm	kcal	gm	kcal
Casein, 80 Mesh	875	3500	50	200	50	200	50	200
DL-Methionine	13.1	0	0.75	0	0.75	0	0.75	0
Corn Starch	20	80	199.6g	692	559	2168	20	80
Maltodextrin 10		0		0	186	600		0
Sucrose	20	80	665.4g	2768	120	692	20	80
Cellulose, BW200	50	0	50	0	50	0	50	0
Soybean Oil	22.2	199.8	22.2	199.8	22.2	199.8	22.2	199.8
Crisco	0	0	0	0	0	0366.7	3300.3	
Mineral AIN Salt Mix S10001	35	0	35	0	35	0	35	0
Vitamin Mix V10001	10	40	10	40	10	40	10	40
Choline Bitartrate	2	0	2	0	2	0	2	0
FD&C Red Dye #40	0	0	0	0	0.1	0	0.1	0
FD&C Blue Dye #1	0	0	0.1	0	0	0	0.1	0
FD&C Yel-low Dye #5	0.1	0	0	0	0	0	0	0
Total	1047.4	3899.8	1035.05	3899.8	1035.05	3899.8	576.85	3900.1

Formulated by E.A. Ulman, Ph.D., Research Diets, Inc, 3/3/94
Chocolate selections are from powdered hot chocolate mix of 4 caloric levels of varying fat and sugar.

weighed back daily to achieve a more precise record of food consumed. The color coding of the powdered diets helped identify the source of spills.

DIETS

The formulas (Table 3.4) for individual macronutrient food cups which were nutritionally balanced were developed in the laboratories of Cornell University (Ulman, personal communication, 1994). These formulations of balanced essential nutrients are used in standard diets for ingestive behavior studies in animal models and have been analyzed for caloric value by bomb calorimetry (Ulman, 1994). The macronutrient choices were individually balanced with vitamin and mineral components so that rats could sustain life if only one food cup source was chosen. The diets were mixed at LSU with standard ICN Biochemical ingredients.

The materials and methods regarding diet selections in each phase were designed to measure hormonal effects on caloric regulation and food choice. Therefore, in phase I the three macronutrient food choices, FAT, carbohydrate (CHO) and protein (PRO) were nutritionally balanced for essential vitamins and minerals (Table 3.1). In phase II the investigation of sweet compared to nonsweet choices of carbohydrate sources (Table 3.2) was divided into sweet AIN-76 which was four times higher in sucrose levels in the non sweet AIN-93 (Reeves, Nielsen, & Fahey, 1993). The sweet food choice was designed to represent simple carbohydrates with a higher sucrose level. The nonsweet food choice was designed to represent complex carbohydrates with the addition of cornstarch, maltodextrin and cellulose (Table 3.4). In phase III the additional measure of chocolate choices was added to the fat, protein, carbohydrate (sweet and nonsweet). The food cups contained

chocolate sources of four caloric levels: High Fat/ High Sugar (HF/ HS), High Fat/ Low Sugar (HF/LS), Low Fat/ High Sugar (LF/LS), Low Fat/ Low Sugar (LF/LS) (Table 3.3). Chocolate sources were four caloric levels of Carnation® Hot Chocolate mix which were mixed with additional hydrogenated shortening and water to provide a non-spill consistency. Low Fat versions, which contained water were calculated for evaporation rates by weighing separate food cups kept on a shelf in the same room. The food cups with chocolate choices did not contain the essential vitamins and minerals that were included in the other macronutrient food cups. The HF/HS was designed to be equivalent to a typical chocolate bar with 33% fat. Carnation Hot Chocolate Powder Mix was used as chocolate source. The Rich Chocolate (HF/HS) was mixed with fat to constitute 5.56 calories per gram. The Sugar Free(HF/LS) was mixed with fat which and was 5.17 calories per gram. The 70 Calorie Mix(HS/LF) was combined with water to form a paste to yield 2.65 calories per gram. Diet Hot Chocolate mix (LF/LS) was mixed with water to yield 1.19 calories per gram. Statistical data was reported in calories not grams.

The diets were mixed in bulk and stored in the freezer until use during the 30 day study. The powdered diets were color coded to more easily detect the source of spills for maximum accuracy in food intake data collection. Liquid food coloring was blended with small amounts of the diet and then incorporated into the bulk mixture. The acceptability of the diet consistency and formulation and four different forms of chocolate were tested in a pilot study.

DATA COLLECTION

Body weight gain was determined daily, and, percentages of body weight were calculated over the 30 day study. Body weight gain and percentage of body weight gain were calculated within phases to show effects of macronutrient choice effects. Food cups were weighed and calculated for mean caloric intake daily, over each 10 day phase and at the conclusion of the 30 day study. Data were collected on an Excel spread sheet with *Balance Link* by Mettler.

Data collection began in phase I (Day 1) with diets of carbohydrate, fat and protein sources (Table 3.1) available ad libitum in separate food containers, with 10-12 animals per hormone treatment group. In phase II (Table 3.2) all groups of rats except the sham group were allowed continuous ad libitum access to four macronutrient choices, protein, fat and carbohydrates (sweet and nonsweet). Food intake was measured to the nearest g between 1000 am and 1100 am daily by weighing food dishes: spillage was caught on paper towels and weighed. In phase III (Table 3.3) the rats, except the sham group, were given four caloric levels of chocolate in addition to the four macronutrient choices (protein, fat, carbohydrate- sweet and nonsweet).

BLOOD SAMPLES

Feeding parameters were followed for a total of 30 days, ten days for each diet phase. Over the next 5 days the rats were fasted 24 hours and then removed from their cages one at a time and sacrificed. Three rats per day were randomly selected from each hormone treatment group to ensure that rats from all groups were randomly sacrificed throughout the entire time period, a total of fifteen rats per day. Rats were anesthetized with ketamine +

xylazine, 3:1 (100 mg/ml, 20 mg/ml), 0.1 ml/100g body weight. Blood samples were taken by cardiac puncture and blood volume recorded. Blood was allowed to clot at room temperature. Sample tubes were spun in centrifuge tubes to separate the serum, which was then pipetted into labeled Eppendorf tubes and immersed in ice for later storage at 4° C or -80° C.

Blood samples were analyzed using radioimmunoassay for ^{125}I estradiol, ^{125}I progesterone and ^{125}I testosterone using Diagnostic Products Corporation Coat-a-Count tubes and reagents (Sitteri & Febres, 1979). The purpose of this analysis was to verify serum hormone levels for documentation of bioactivity. The procedure is based on the competitive inhibition of binding radioactively labeled hormones to a specific antibody. Concentrations of hormones in the 10 - 12 picogram range can be measured by this technique. Cholesterol and HDL cholesterol measurements were determined using Sigma Kits for colorimetric methods (Perkin-Elmer Lamba 1 UV Vis spectrophotometer) (Friedewald, et al, 1972).

CARCASS COMPOSITION

At the conclusion of the study the rats were sacrificed according to the protocol of the animal care committee (LSU Internal Review Board Regulations, 1986). The hearts, livers and uteri were harvested, weighed and frozen for further analysis.

Carcass composition of the five treatment groups was determined and reported in terms of percentage of carcass weight of lipid, protein, and water (Maggio, et al., 1984). Eviscerated carcasses, autoclaved under pressure, and homogenized using the Brinkman polytron/3500. Aliquots of the carcass homogenates were analyzed for the determination of nitrogen according to the

micro-Kjeldahl method, lipid according to the ether extraction method and moisture by drying to a constant weight at 100 degrees C. Weights and composition of the excised samples were used in the final calculations. Additionally, fat extraction procedures were used to analyze fatty contents of the livers using the same method described in total body composition analysis. The Soctex Instrument made by the Tecator Subsidy of the A Powerstep Analytical Co. Indianapolis, Indiana was used in the fat extraction analysis. Moisture analysis was conducted in a drying oven made by Sanyo Corporation, Sussex, England. Micro-Kjeldahl measures for protein were conducted on the autoanalyzer model BD-20 (Technicon Instruments Corp.) according to procedure 976.06 in the *Official Methods of Analysis of the Association of Official Analytical Chemists, (1990).*

STATISTICAL METHODOLOGY

Prior to implantation, rats were randomly divided into five groups. There were no significant differences in starting body weights between rats assigned to the five treatment groups and no significant difference between groups. Hormone implants were surgically implanted in random order. The mean body weight of the group of 55 rats was 268.1±3.1 on the day the data collection began. However, groups did not weigh the same after the two day recovery period when the first body weight and food intake data were collected and recorded. On day one of the data collection the beginning mean body weights were: S: 272±8.2, OV: 266±7.7, E: 258±5.9, E&P: 266.2±6.5, P: 277±3.5.

For analyses of food intake (caloric, macronutrient and chocolate), the experiment was divided into three, ten day periods of each diet protocol for a

total of 30 days. The food intake and body weight data were divided into three phases. For each of these periods, one-way analyses of variance were used to determine hormone treatment effects on food intake and body weight data. One-way analysis of variance was also used to determine hormonal effects on different caloric macronutrient intake. Scheffé's multiple comparison procedure was used to determine the differences among the treatments. Additionally, linear group contrasts were used to determine the differences between the four ovariectomized groups (E, E&P, and P) vs nonhormone (OV), between estrogen (E, E&P) vs nonestrogen (OV, P) and between progesterone (E&P, P) vs nonprogesterone (OV, E) in clusters of treatments. Phase I and phase II differed only by the inclusion of the sweet/nonsweet investigation of the carbohydrate macronutrient group.

In Phase III, 4 caloric levels of chocolate were added to the food choices. In the analysis of caloric intake, foods were first converted from weight consumed (in grams) to caloric value. From those calculations, conversion ratios (CR) of body weight gained per 1000 calories consumed were determined (CR = grams of body weight increase divided by caloric intake X 1000). Conversion ratios were calculated for each phase and over the 30 day period.

One way analysis of variance was utilized to determine the effects of hormone treatments on total body weight gain, % body weight gain, total caloric intake, % of caloric intake from individual macronutrients and total individual macronutrient intake, food intake (macronutrient and chocolate choices), body composition, cholesterol, serum hormone levels, liver, heart and uterine weights, weight to length ratios, fat content of livers, and caloric

conversion ratios. Data were collected with the use of Microsoft Excel spreadsheets and analyzed with Superanova Statistical software, Abacus Concepts (Gagnon, 1989). One way ANOVA with contrasts and ANOVA factorial analysis with two independent variables (level of estrogen and progesterone) were used. ANOVA percentages were used to statistically compare the data obtained as percentages. Phase I analysis was conducted on all five groups since they received the same food choices. Phase II and III were analyzed by including the four ovariectomized groups receiving all food choices. Data for the total study reflected all three phases in combined and separate analysis. Both four and five group treatments (S for comparison) were included in statistical analysis.

PILOT STUDY

A pilot study was conducted to test macronutrient mixtures and chocolate (chocolate syrup, chocolate chips, chocolate bars and chocolate paste made from hot chocolate mix) for palatability and form of presentation to minimize spillage and loss (Ulman, personal communication, 1994). The chocolate paste was found to be the most acceptable form to the rats. The surgical administration of the hormone by silastic tubing implant was also piloted (Richard, 1986). This procedure provided continuous hormone release without injections (Smith and Walsh, 1976). All laboratory procedures and statistical modeling were examined and evaluated during the pilot testing period and by consulting scientists directly involved in endocrinology and eating behavior research.

CHAPTER 4 RESULTS AND DISCUSSION

In this section, specific p values for statistical comparisons will be found in tables or appendixes (denoted by page number). Statistical techniques used for data mentioned in the text will also be denoted in parentheses. In Phase I, data in the tables are based on ANOVA with five treatment groups and five group ANOVA percentages. Analyses other than five treatment group ANOVA are shown in the appendixes, for example four group ANOVA, percentages, group contrasts and factorial analysis. Phase II and III are based on 4 group ANOVA, excluding the sham. The sham treatment group should be judged as a comparison group which was maintained on the three macronutrients throughout the study. Abbreviations for treatments are as follows: ovaries left in tact (sham), S; ovariectomy with no hormone treatment, OV; estradiol, E; estradiol and progesterone, E&P; progesterone, P; all endogenous (S) and exogenous hormone treatments, H. All summary figures appear in Appendix F (p208-224). Any statement of significance (reported in 3 digits) made in the text means that p was less than 0.05. Actual computer generated numbers in tables are reported (p=) for the purpose of conversion to relative risk ratio calculations for use in future medical and physiological publications.

RESULTS OF PHASE I: OVERVIEW

In Phase I, three choices of macronutrients were presented: fat, carbohydrate and protein in all five treatment groups.

BODY WEIGHT

Body weight gain (total & %) among groups was affected by hormone treatment but caloric intakes were not (Table 4.1A, Figure 1.0 p208 and 4

TABLE 4.1A PHASE I - BODY WEIGHT GAIN AND TOTAL CALORIC INTAKE

Treatment	Sham	Ovariec-tomized	OV + 17β Estradiol	OV + 17β Estradiol + Proges-terone	OV + Proges-terone	ANOVA p value
Number of Animals (n=)	12	10	11	12	10	
Original Weight of Animals	272•	264	258	271	275	p=.388
Total Weight Gain (g)	20.7± 3.1 (a)	24.4± 2.3 (a)	6.4±2.0 (b)	14.7± 2.7 (a,b)	27.6± 5.0 (a)	p=.001
% of gain	7.8% (a)	9.2% (a)	2.6% (b)	5.5%(a,b)	10.0%(a)	p=.001
Total Caloric Intake	831 ± 42.0	898 ± 47.8	773 ± 35.5	831 ± 50.5	883 ± 40.2	p=.314
% of total	38.7%	32.1%	31.0%	31.4%	32.2%	
Caloric Conversion Ratio*	24.6±3.5 (a)	27.8±2.8 (a)	8.8±2.8 (b)	17.0±2.9 (a,b)	29.8±4.5 (a)	p=.001

• Initial mean body weight of the five groups on day one of data collection was 268.1.

Values are means ± SE. Means in the same row with the different superscripts are significantly different from each other by Scheffé's S procedure for post-hoc comparisons.
% of gain is for this 10 day period
% of total caloric intake shows percent of caloric total (phase I, II, III) consumed in phase I
* Caloric conversion ratio formula (CR): Grams of body weight increase divided by caloric intake X 1000.

ANOVA p192). OV treated animals implanted with E gained less weight than the S, OV, and P (total & % Table 4.1A). The progesterone treated animals were the only hormone group significantly higher than the estrogen group (total & % Table 4.1A). Initial body weight differences were examined by ANOVA at the beginning of the study. There were no significant differences (Table 4.1A ANOVA initial body weight p 193). Group contrasts showed that OV animals gained more weight (total & %) than the hormone treated animals and E was significantly lower by 59% than NonE (4 group ANOVA p192 total & % Table 4.1B). The estrogen effect on decreased body weight gain(4 group ANOVA factorial p193) was greater than E&P or P.

CALORIC INTAKE

There was a trend (p=0.052) for decreased caloric intake for the E animals compared to Non E animals (Total Table 4.1B). A trend (p=.054) was also shown (4 group ANOVA factorial p193) with four group analysis.

CALORIC CONVERSION RATIO

The conversion of calories consumed into body weight gain was significantly lower for the E group compared to S, OV and P (Total Table 4.1A Total 4 group ANOVA p193, 4 group ANOVA factorial p194). Hormone treatments significantly lowered caloric conversion ratios compared to the OV animals with no hormone in group contrasts and E significantly lowered caloric conversion ratios compared to Non E (4 group ANOVA p194).

TOTAL FAT

When the distribution of macronutrients consumed (Table 4.1C) was examined there were no significant among treatment differences in

TABLE 4.1B PHASE I - GROUP CONTRASTS TOTAL WEIGHT GAIN AND TOTAL FOOD INTAKE

Tmt	n	Total Weight Gain*		p value	Total Caloric Intake (kcal)*		p value	Caloric Conversion Ratio	p value
			%	g		%			
		g	Wt.	%	kcal				
S vs	12	20.7 ±3.1	7.8 ±1.3	p=.211	831.0 ±42.0	37.9 ±1.2	p=.971	24.6 ±3.5	p=.756
H	33	15.8 ±3.3	5.9 ±.9	%p=.191	827.6 ±42.1	31.4 ±.6		18.4 ±3.4	
OV vs	10	24.4 ±2.3	9.2 ±.8	p=.035	898.0 ±47.8	32.2 ±.9	p=.194	27.8 ±8.9	p=.025
H	33	15.8 ±3.3	5.9 ±.9	%p=.029	827.6 ±42.1	31.4 ±.6		18.5 ±3.4	
E vs	23	10.7 ±1.9	4.1 ±.7	p=.001	803.5 ±31.3	31.1 ±.7	p=.052	12.9 ±2.8	p=.001
Non E	20	26.0 ±2.7	9.6 ±1.	%p=.001	890.7 ±30.4	32.2 ±.7		28.8 ±3.7	
P vs	22	20.5 ±3.0	7.5 ±1.1	p=.077	854.9 ±32.8	31.6 ±.8	p=.632	23.4 ±3.7	p=.267
Non P	21	15.0 ±2.5	5.7 ±.9	%p=.071	832.8 ±31.8	31.6 ±.7		18.3 ±2.8	

Sham = S
Hormones = E, P, E&P
Ovariectomized = OV, no hormones
Estrogen = E, E&P
Non Estrogen = OV, P
Progesterone = P, E&P
Non progesterone = OV, E

TABLE 4.1C PHASE I - MACRONUTRIENT FOOD CHOICES IN KCAL

Treatment	Sham	Ovariec-tomized	OV + 17β Estradiol	OV + 17β Estradiol and Pro-gesterone	OV + Proges-terone	ANOVA p value
n	12	10	11	12	10	
Total Intake of Carbo-hydrate (Calories)	368.6 ± 43.1 (a,b)	542.2 ± 55.4 (a)	372.1 ± 40.6 (a,b)	314.8 ± 36.2 (b)	413.2 ± 33.6 (a,b)	p=.007
% of total	45.5% (a,b)	59.4% (a)	47.5% (a,b)	38.0% (b)	47.8% (a,b)	p=.027
Total Intake of Protein (Calories)	34.4± 6.2 (a)	84.6 ± 22.8 (a,b)	87.8 ± 17.5 (a,b)	132 ± 16.1 (b)	66.3 ± 16.8 (a,b)	p=.001
% of total	4.2% (a)	9.3% (a,b)	11.7% (a,b)	16.2% (b)	7.6% (a,b)	p=.001
Total Intake of Fat (Calories)	428.0 ± 58.6	271.5 ± 41.8	313.3 ± 35.5	384.4 ± 41.6	403.6 ± 57.5	p=.144
% of total	50.3%	31.3%	40.8%	45.8%	44.7%	p=.117

Values are means ± SE. Means in the same row with the different superscripts are significantly different from each other by Scheffé's S procedure for post-hoc comparisons (p=.05)
% is for this 10 day period

calories from fat. However, a P effect was observed on total fat intake compared to NonP (Total Table 4.1D). Additionally, when fat % group contrasts (Table 4.1D) were conducted, all hormone treatments significantly increased fat intake compared to OV which did not receive hormones (Figure 2.0, 3.0 page 209-210). These findings were confirmed by factorial analysis (ANOVA factorial p194). A significant P effect on FAT consumption was evident which means that P alone had a greater effect on FAT intake than E alone or E combined with P.

TOTAL PROTEIN

E&P treated animals consumed three times as much protein as the S animals (Total & % Table 4.1C). In both total and percent group contrasts, S consumed significantly less protein than H, and E consumed significantly more protein than NonE (Table 4.1 D).

TOTAL CARBOHYDRATE

E&P treatment consumed less CHO compared to the OV group in both total and percent (Table 4.1C). Similarly, hormone treatments also decreased the total intake of CHO in rats as contrasted with ovariectomized animals not receiving hormones (Total & % Table 4.1D). Significant differences were also observed in E vs. NonE and P vs NonP (Total & %). Also, factorial analysis provides evidence that both estrogen and progesterone significantly affect CHO intake (4 group ANOVA factorial p195).

DISCUSSION OF PHASE I

In phase I, the effect of estrogen on decreased body weight gain was evident with all statistical methods of analysis. The question of the effects of

TABLE 4.1D PHASE I - GROUP CONTRASTS FOR TOTAL CARBOHYDRATE, PROTEIN AND FAT

Treatment	n	Carbohydrate*		p value	Protein*		p value	Fat*		p value
				g			g			g
		g	%	%	g	%	%	g	%	%
S	12	369.0	45.5	p=.970	34.4	4.3	p=.015	428.0	50.3	p=.263
vs		±43.1	±5.3		±6.23	±.8		±58.6	±5.3	
H	33	363.7	4.1	%p=.890	97.4	12.1	%p=.001	367.0	43.8	%p=.197
		±36.8	±2.4		±16.8	±1.3		±44.9	±2.7	
OV	10	542.0	59.4	p=.001	84.6	9.3	p=.583	272.0	31.3	p=.104
vs		±55.4	±4.0		±22.8	±2.4		±41.8	±5.2	
H	33	363.7	14.0	%p=.006	97.4	12.1	%p=.496	367.0	43.8	%p=.044
		±36.8	±2.4		±16.8	±1.3		±44.9	±2.7	
E	23	342.2	42.5	p=.03	110.9	14.0	p=.042	350.0	43.4	p=.818
vs		±27.1	±2.9		±12.5	±1.6		±28.0	±3.3	
Non E	20	478.0	53.6	%p=.019	75.5	8.4	%p=.008	338.0	38.0	%p=.719
		±34.8	±14.1		±13.9	±6.5		±37.8	±3.9	
P	22	359.6	42.4	p=.03	102.2	12.3	p=.434	393.0	45.3	p=.043
vs		±26.6	±3.2		±3.4	±1.6		±33.8	±3.5	
Non P	21	453.1	53.2	%p=.022	86.2	10.5	%p=.396	293.0	36.3	%p=.057
		±38.1	±2.9		±13.9	±7.8		±26.9	±3.5	

Sham = S
Hormones = E, P, E&P
Ovariectomized = OV, no hormones
Estrogen = E, E&P
Non Estrogen = OV, P
Progesterone = P E&P
Non progesterone = OV, E

exogenous female sex hormones on additional body weight was answered. Estrogen replacement curbed the body weight gain which occurred as the result of the ovariectomy. The significant differences in body weight were not based on caloric intake although a trend for an estrogen effect was observed. This raised the question of cause and effect which led to the calculation of caloric conversion ratios. Estrogen appeared to cause a decrease in the efficiency rate at which calories are converted into body weight which is supported by Stern et. at. (1974), and Storlein et al., (1979) OV converted calories more readily into body weight than all hormone treatments. Non E treated animals converted more efficiently than those implanted with estrogen.

Hormones may also affect the choice of macronutrients. Animals implanted with P consumed significantly more FAT than Non P implanted animals. When grouped together, H treated animals consumed more FAT than animals in the OV treatment group which received no hormones. This supports the hypothesis that FAT intake is higher in the luteal phase of the normally cycling female when P levels are elevated. The combination of E&P appeared to motivate PRO consumption when compared to S, which agrees with Wurtman, et al. (1992). Factorial analysis revealed that E in the presence of P is more effective in stimulating PRO consumption than either hormone individually. With group contrasts, hormonally treated animals and those implanted specifically with E chose more P than S and Non E treatments. The compensation or preference for calories from another macronutrient group by the OV animals, was from CHO. They chose more CHO than the E&P treated animals. In both total and percent in group contrasts, H, E and P suppressed CHO consumption to a greater degree than OV, Non E and Non P

respectively. These data imply that hormone replacement may suppress the craving for highly refined carbohydrate snack foods.

It has been known for years that exogenous estrogens exert anorectic effects in rodents (Wade, 1976; Wade, Gray, & Bartness, 1985). Reduced food intake has been observed following the preovulatory surge of estradiol in younger animals that occurs either at proestrus or at the end of the follicular phase, depending on the species. In addition, the anorectic effects of estrogens (Kemnitz, et al. 1989; Haarbo, et al, 1991; Geary et al, 1994) have also been emphasized in ovariectomized animals in which estrogen replacement therapies reduce the effect of removal of the ovaries. In rats, generally estradiol decreases the intake and increases the expenditure of energy, which results in decreased body fat stores in the younger rat models. Progesterone reverses nearly all of these effects of estradiol and thus, promotes fat storage in rats (Poucher, Tobin, 1985). In a series of experiments, Gray & Wade, (1981) reported increased food intake, body weight, and carcass adiposity in OV rats treated with progesterone but not in OV rats treated with estradiol benzoate. However, it must be noted that in Phase I in the present study, the group without ovariectomy and no exogenous hormones (S) responded similarly to the progesterone group. The above studies agree with the findings of the present study in the postbreeder rat in body weight but not in caloric intake and adiposity. No significant differences were observed in body composition in the present study in the older models.

The young female Sprague-Dawley rat model has been used extensively in much of the previously reported work on gonadal female sex hormones. In earlier studies in younger rats (Gray & Greenwood, 1984) the

injection of progesterone alone, to OV had no additional effect on food intake or body weight beyond that observed in OV only (Blaustein & Wade, 1977; Wade & Gray, 1979). This agrees with the present study. Additionally, in his review of effects of gonadal hormones, Wade & Schneider (1992) reported that administration of progesterone to either estrogen-primed or gonadally intact adult rats led to increased food intake and an increased rate of body weight gain. This could parallel the luteal phase in the normally cycling female (Dalvitt-McPhillips, 1983). Estrogen alone or estrogen combined with progesterone as shown in the follicular phase in the cycling female suppressed body weight gain. An increased level of maturity combined with hormonal status may provide the differences observed in the present study. Additional research should be conducted on the aging model to examine what has earlier been implicated as a reflection of gonadal influence of food intake and body weight.

Fluctuations in the rate of conversion of caloric intake into body weight may be seen under the influence of exogenous female sex hormones both in the rodent and the human female and in future studies. The Sprague-Dawley rat appears to be a valuable model in preliminary work to show trends and parameters not scientifically measurable in the human. Other aging strains and species of animals should also be tested.

Galletti and Klopper (1964) found that when mature female rats were given 3.14 mg of progesterone daily for 20 days they showed a greater body weight gain than controls. Carcass analysis revealed this extra weight to be due to an increased fat deposition. Immature females did not gain weight faster than controls and showed no enhanced fat deposition. They reported

that when adult female rats were castrated they gained weight faster than intact animals but treating such castrated animals with progesterone did not cause an additional weight increase beyond the OV effect. The average daily weight gain in the P treated animals was 2.33g daily compared to the intact controls which gained 1.56g body weight per day. The additional body weight in the postbreeder OV and P treated animals in the present study reveal the same results for body weight; however, no significant differences in adiposity were observed.

Differences have also been examined in lean compared to obese rats. Gray and Greenwood (1984) injected 5 μg of estradiol benzoate (EB) in OV rats for five days, which resulted in decreases in the rate of body weight gain in both lean and obese Zucker rats. Additionally, EB administration resulted in significant induction of progestin binding sites in both neural and adipose tissues. However, EB treatment significantly decreased lipoprotein lipase activity in adipose tissue from lean but not obese Zucker rats. Their research shows that the presence of receptor sites for estrogens and the receptivity of the sites for progestins can be demonstrated in both adipose and uterine tissues. This suggests that the enzymatic changes that are observed in these tissues following hormone administration may be receptor mediated and that the original state of lean compared to obese may reveal very different results. These mechanisms may also help to explain the results of the present study with regard to differences observed in E and P treatments.

The effects of estrogenic stimulation (not progesterone) on diet selection were examined by Bartness & Waldbillig (1984) on intact, OV and E treated animals. The FAT, PRO and CHO levels of laboratory chow were

manipulated. OV treatment was associated with the nearly exclusive choice of higher caloric intake which was decreased in the E treated animals. Total caloric intake and body weight did not vary across the estrus (follicular) cycle. However diet selection did vary. Fat intake increased; CHO and to a lesser extent, protein intake decreased during estrus when estrogen levels are elevated. An opposite diet selection occurred during diestrus (luteal). OV treated animals (no endogenous E & P) chose progressive increases in CHO and protein intake, with concurrent decreases in fat consumption. The E treated animals partially reversed this diet selection profile demonstrating estrogenic reduction in CHO intake with standard high-CHO commercial diets. In addition, an increase in fat intake during estrogenic stimulation was found. This supports the results of the present study with regard to food intake and body weight patterns in the OV and E treatments as a reflection of estrus.

The mechanistic effects of cyclic estradiol replacement on satiety and cholecystokinin (CCK-8) with subsequent resulting body weight gain in ovariectomized rats (Geary, et. al, 1994) were recently reported. Estradiol significantly increased CCK-8's inhibitory effect on sucrose intake. In contrast, progesterone alone or in combination with estradiol did not consistently influence the satiating potency of CCK-8. Potentiation of the satiating effect of CCK released from the small intestine by ingested food may be one of the mechanisms by which food intake decreases during the period of high estrogen concentration in the estrus cycle. During the 10 week study the ovariectomized rats treated only with the sesame oil vehicle gained about 60 g more than the estradiol-treated rats. These results are supported by treatment

and body weight data found in Phase I of the present study and may help to explain estrogen mechanisms.

The physiological phenomena which affected the outcomes in the aging female rat in phase I of this study with no significant differences in caloric intake (Figure 3.0) are not currently known. In Phase I, all animals received the same three macronutrient food choices. Estrogen replacement caused reduced weight gain, however, progesterone failed to suppress body weight gain compared to E treated animals. Additionally, ovariectomy and hormone treatments significantly altered preferences for macronutrient food choices.

Contrary to previously reported studies (Wade, 1976; Wade & Gray 1979), the increase in body weight in the present study was not accompanied by additional caloric intake in Phase I. However a trend was observed ($p=.052$) for lower caloric intake in the E treatment. Progesterone, which did not significantly increase body weight and food intake in the intact female rat (Wade & Gray, 1979) was found to increase body weight in the aging model in phase I when compared to estradiol treated animals. This supports the conclusions of other previous studies in the younger model (Hervey & Hervey, 1967) which suggests that both ovariectomy and progesterone increase food intake and body weight. Galleti and Klopper (1964) reported that progesterone appears to lead to the laying down of fat, and that this can come about in one of three ways. Either the animals take in more food, or make more body fat out of the same amount of food by more efficient use of foodstuffs or else energy output is reduced by the sedative effect of progesterone. This view (of progesterone effects) in immature animals is supported by the fact that a progesterone effect was also observed in mature animals in Phase I and Total

Study analysis in the present research. Possibly the effect of progesterone on weight gain in Phase I in adult female animals arises from the suppression of estrus at which time the animals are very active.

The obvious question becomes, was increased caloric expenditure a factor? Although the effects of female sex hormones on body weight in young rats are rather well documented (Hervey & Hervey, 1967; Wade, 1976; Wade & Gray, 1979), there is little information in the literature detailing the mechanisms through which these hormones act on the regulation of energy balance. According to previous reports, estradiol increases energy expenditure by playing a role in increasing physical activity (Rodier, 1971; Wade & Gray, 1979). Richard (1986) has suggested that when food intake is kept constant, ovarian hormones affect energy balance by altering energy expenditure. In the current study, exercise was not measured. However, empirical observations indicate that an increase in body weight gain may be due to decreased activity, lack of endogenous hormones or hormone induced lethargy. A pronounced lethargy and hostility were observed in P treated animals. Voluntary exercise in the aging female rat should be measured in future research studies.

The mechanisms responsible for hormonally induced increases in food intake in ovariectomized rodents are also poorly understood. Tarttelin (1973) has shown that the effect of the ovariectomy diminishes after 4 weeks. This provides evidence of a potential existing mechanism that results in altered caloric conversion ratios shown in the present study. The reported increase in food intake observed after ovariectomy (Hervey & Hervey, 1967) is probably due to the removal of the circulating estrogens which are known to have an

anorectic effect. The most interesting concepts surround a messenger system which sends information from the digestive system to the brain. Neural messengers and the mechanisms involved are currently under investigation to determine hormonal effects on food intake regulation in the female rat influenced by exogenous female sex hormones (Geary, et al, 1994; Butera, 1995 personal communication). No conclusive evidence has been published. The significant differences in caloric conversion ratios in the present study suggest that there may be metabolic alterations stimulated by exogenous female sex hormones yet unreported.

In a review of known or hypothesized potential mechanisms, Wade and Schneider (1992) reported that a transience could be related to estradiol-induced shifts in a hypothetical weight set-point. Changes in food intake would in this case persist only until body weight is brought into line with a new regulated level (Harris, 1990). A more useful working hypothesis may be that ovarian steroids act centrally or peripherally to perturb the balance between the storage and mobilization of metabolic fuels (e.g., via changes in autonomic activity, insulin and counter-regulatory hormones, adiopocyte enzyme activities, hepatic fuel metabolism, etc.). Food intake could change in response to changes in fuel availability that result from these alterations in fuel partitioning. Once a new equilibrium is established, body fuel stores would then stabilize at a new level, and fuel availability (and, consequently, food intake) would revert to the previous values. The questions must then be, is this phenomenon inevitable in mid life or following an ovariectomy? Can or should it be manipulated? Perhaps the current practice of injecting replacement hormone into females immediately following the hysterectomy helps to prevent

this added body weight in the human female. That investigation in the human has not been reported (Hayes, personal communication, 1995).

Ovarian steroids affect other food-related behaviors in rats. Ovariectomy causes a transient increase in the hoarding of food, and treatment with estradiol suppresses food hoarding in ovariectimized rats (Coling & Herberg, 1982). Various laboratories have used different protocols and different macronutrient sources, generally presented with sucrose in water bottles. It is conceivable that ovarian steroids primarily affect hedonic responses of rats to taste stimuli, affecting the animals' response to differences in the relative palatability of the available macronutrients. Indeed, ovarian steroids have significant effects on rats' preferences for nonnutritive sweet and bitter tastes, and on electrophysiological responses to tastes in the brain stem which is a major relay for taste information. Experimental manipulations of circulating gonadal steroid levels indicate that many of these changes in energy balance with reproductive status are due to the actions of these hormones, both in the brain and on nonneural peripheral tissues (DiLorenzo & Monroe, 1989; Wade & Zucker, 1969, 1970; Zucker, 1969). Little else is known about the neural circuitry mediating the actions of estradiol on food intake (Butera, personal communication,1995).

There are numerous interconnected neural structures which contain estrogen receptors and which: 1) receive the chemo-sensory and visceral inputs; 2) integrate this information with other sensory cues; and 3) modulate the autonomic, hormonal, and behavioral responses to this information (Wade & Schneider, 1992). These structures within the brain include the area postrema and the nucleus of the solitary tracts, the mid brain central gray, the

lateral parabrachial nucleus, the central nucleus of the amygdala, the bed nucleus of the stria terminallis, the lateral hypothalamus, the paraventricular nucleus, and the ventromedial hypothalamus (Loewy, 1990; Pfaff & Keiner, 1973; Simerly, et al, 1990).

Psychologists, physiologists, biochemists and molecular biologists are attempting to measure and report these mechanisms. Wade (1987) stated that one strategy would be to manipulate energy balance with estradiol treatments and measure changes in neuronal function, such as *c-fos* activity, in brain regions known to be associated with regulation of feeding and body weight. Indeed, estradiol treatment has been shown to alter *c-fos* expression in rat brain (Hoffman, et al, 1990; Insel, 1990). An obvious problem with this approach is that ovarian steroids act in the brain to affect a wide variety of behaviors and physiological responses, not just those affecting energy balance. In addition, there is substantial anatomical overlap of the neural systems controlling energy balance and other hormone-sensitive functions, making it difficult to relate hormone-induced neuronal changes to any particular behavior or physiological endpoint (Wade & Blaustein, 1978). However, it might be possible to avoid, or at least minimize, this problem by using nonsteroidal antiestrogens. These compounds (e.g., tamoxifen, nafoxidine, MER-25) block most of the neuroendocrine actions of estradiol, including running wheel activity, estrus behavior, maternal behavior, and gonadotrophin secretion (Andieh, et al, 1987; Bowman, et al, 1983; Gerall, et al, 1973; Komisaruk & Beyer, 1972; Labhsetwar, 1970; Roy, et al, 1979; Roy & Wade, 1975, 1977; Wade & Blaustein, 1978). On the other hand, nonsteroidal antiestrogens mimic the actions of estradiol and reduce food intake and body

weight in ovariectomized rats; they are completely devoid of antagonistic activity on these measures (Bowman, et al, 1981, 1982; Donohoe & Stevens, 1982; Gray & Wade,1981; Roy & Wade, 1977; Wade & Blaustein, 1978).

Although estradiol-induced decreases in food intake undoubtedly contribute to the observed decreased body weight gain, pair-feeding experiments indicate that behavioral changes are neither necessary nor sufficient to cause weight loss. Estradiol-treated rats fail to gain weight even if they do not undereat (Kennedy, 1953; Thomas, et al, 1986; Wade, 1974; Zucker, 1972). However, in another study the hypophagia normally seen during estradiol treatment was not sufficient to cause weight losses in vehicle-treated, ovariectomized rats (Wade and Gray, 1979). Finally, the majority of the studies reported confirm that ovarictomized rats gain weight and fatten whether or not they are permitted to overeat (Bartness & Waldbillig, 1984; Landau & Zucker, 1976; Mook, et al, 1972; Roy & Wade, 1977; Thomas, et al, 1986). Thus, it is clear that estradiol and ovariectomy both have significant effects on the postingestive handling and utilization of metabolic fuels. In the present study, significant differences in treatment effects of caloric conversions ratios are evidence of these phenomonen in the aging rat model.

Earlier research reported on younger animals by Wade, Gray and Bartness (1985) indicated that exogenous female sex hormones, in addition to the well-known actions on reproductive physiology and behavior, have been reported to affect a wide variety of nonreproductive functions, including the metabolic processes that regulate energy balance, body weight, and body composition. They have reported that among female rats there are significant fluctuations in behavioral and physiological controls of energy balance with

changes in reproductive conditions such as puberty, ovulatory cycles, pregnancy, lactation, and reproductive state. In addition, they have shown with experimental manipulations of circulating gonadal hormones that many of these changes in energy balance with reproductive status are due to the actions of female sex hormones, both in the brain and on non-neural peripheral tissues. This work in the mature rat model remains to be done.

The following are some examples of how ovarian steroids act in some metabolically important organs, such as liver and adipose tissue, to adjust the metabolism and partitioning of calories. After food is absorbed from the gut, most of it enters the splanchnic circulation and is carried to the liver. Substrates from other sources (e.g., fatty acids and glycerol from adipose tissue, amino acids from muscle) are also carried to the liver. These fuels may simply pass through the liver on their way to other organs or they can be oxidized, stored as glycogen, or converted to other forms (e.g., gluconeogenesis, lipogenesis, ketogenesis).

Ovarian steroids have a number of important effects on hepatic handling of metabolic fuels. For example, ovariectomy increases hepatic gluconeogenesis and reduces tissue glycogen content, and these changes are reversed by treatment with estradiol alone (Ahmed-Sorour & Bailey, 1981; Matute & Kalkhoff, 1973; Sladek, 1974). These effects in both human and rats could be due to direct hepatic actions of estradiol, because the liver is known to be a target tissue for estrogens (Aten, et al, 1980; Eisenfled, et al, 1977). In addition, estradiol could act indirectly by modulating hepatic responsiveness to glucoregulatory hormones, or by altering the secretion of these hormones via direct or indirect actions on the pancreas (Ahmed-Sorour & Bailey, 1980;

Bailey & Ahmed-Sorour, 1980; Faure, et al, 1979; Mandour, et al, 1977; Storlien, et al, 1979; Tesone, et al, 1979; Thomas, et al, 1986).

Manipulations of ovarian steroid levels also affect hepatic lipid metabolism. Ovariectomy decreased hepatic lipogenesis and triglyceride and lipoprotein production; sustained estradiol replacement therapy reverses these changes. However, careful examination of the time-course of these effects also indicates that estradiol treatment had biphasic effects on hepatic lipid metabolism (Edens & Wade, 1983). In the short term, estradiol inhibits hepatic lipogenesis and triglyceride release, but with longer treatment times, these functions are stimulated (Eisenfeld, et al, 1977; Gray & Greenwood, 1982; Kenagy, et al, 1981; Kim & Kalkhoff, 1975, 1978; Ramirez, 1980). Therefore, it is not really clear from the existing literature, how the relatively rapid (day-to-day, hour-to-hour) changes in circulating levels of ovarian steroids that are seen across ovulatory cycles affect hepatic lipid metabolism in rats. Clearly cholesterol, LDL, and HDL were affected in the PEPI subjects who were postmenopausal females taking exogenous female sex hormones (PEPI Writing Group, 1995).

Ovarian steroids have striking effects on the storage of calories in adipose tissue. They do this by altering the activities of the enzymes that control both the uptake and release of metabolic fuels by adipocytes. In rats, ovariectomy has been shown to increase body fat stores whereas treatment of ovarictomized animals with estradiol decreases body weight and fat content (Leshner & Collier, 1973; Wade, Gray, and Bartness, 1985; Wade & Gray, 1979). Percentage of body composition is not indicated, but should be reported in future studies.

Estradiol probably acts both centrally and peripherally to alter adipose tissue fuel metabolism. Rat adipose tissues contain steroid-specific, high-affinity, limited capacity receptors for both estrogens and progestins that are similar to the receptors in other steroid target tissues (Gray, et al, 1981; Gray & Wade, 1979; Pedersen, et al, 1991; Wade & Gray, 1979). These findings support the possibility that estradiol could act directly on adipocytes to alter enzyme activities (Wade & Schneider, 1992).

It is conceivable that estradiol might act on other peripheral tissues, such as the liver, to alter adipose tissue enzyme activities indirectly (Wade & Gray, 1979). For example, estradiol can act directly on the liver to modulate the production and release of certain apoproteins which act as cofactors for adipose tissue lipoprotein lipase (Kim & Kalkhoff, 1978).

The mechanisms in the brain have been more extensively examined. Estradiol may act in the brain to modulate adipose tissue metabolism via either hormonal (Wade & Schneider, 1992) or autonomic outputs. Cutting the sympathetic nerves to retroperitoneal white adipose tissue significantly attenuates the estradiol-induced fat pad weight loss (Lazzarini & Wade, 1988 a). This reduction of fat pad responsiveness to estradiol is not due to a decrease in estrogen receptor concentration. Instead estradiol seems to increase the activity of the sympathetic nerves to the retroperitoneal fat pads, but not those to the heart (Lazzarini & Wade, 1988b). An estradiol-induced increase in release of catecholamines from sympathetic terminals would stimulate lipolysis. It is likely that estradiol acts centrally to stimulate sympathetic activity, since many of the neural areas that control autonomic activity are also target sites for estradiol (Lowey, 1990; Pfaff & Keiner, 1973;

Simerly, 1990). Thus, it appears that estradiol enhances lipolysis by acting both in the brain to stimulate sympathetic activity (and consequently norepinepherine release in adipose tissue) and in adipose tissue to increase lipolytic sensitivity to catecholamines (Benoit, et al, 1982; Hansen, et al, 1980; Lacasa, et al, 1991; Lazzarini & Wade, 1988a).

Estradiol may affect adipose tissue physiology indirectly, via the actions of other hormones. Estradiol alters the secretion of hormones, which may have important effects on metabolism and adipose tissue (Faure, et al, 1983; Storlien, et al, 1979; Wade & Gray, 1979). It is unlikely that estradiol-induced changes in insulin secretion play a critical role in the effects of estradiol on energy balance. Estradiol treatment increases circulating levels of insulin, but this should increase, rather than decrease body fat stores. In addition, the actions of ovariectomy and estradiol replacement on body weight are not impaired in streptozotocin-diabetic rats given controlled amounts of insulin (Faure & Sutter-Dub, 1979; Faure, et al, 1983; Mandour, et al, 1977; Storlien, et al, 1979; Thomas, et al, 1986).

Estradiol increases the expenditure of metabolic energy via changes in both regulatory behaviors and metabolic processes. Ovariectomy decreases and estradiol treatment increases metabolic rate and thermogenesis in rats. This estradiol-induced increase in heat production is accompanied by a rise in dry heat loss, so that changes in core temperature are absorbed, or at least inconsistent. That is, the extra heat is dissipated as fast as it is generated, and estradiol increases heat flux without affecting the body temperature substantially (Wade & Schneider, 1992).

Although thermoregulatory mechanisms often signal the beginning of menopause, very little is known about the effects of ovarian steroids on energy balance in mature women. Food intake and metabolism are known to vary with reproductive condition, including menstrual cycles, pregnancy and menopause (Wade & Schneider, 1992). Virtually nothing is known about the effects of exogenous human female sex hormones on energy balance. We know only that in women of a wide variance of ages who are taking exogenous female sex hormones, body weight changes occur. More research clearly needs to be conducted.

During hormonal shifts throughout the life cycle, females are often aware of changes which can be felt physiologically in feelings of fullness, due to edema and perceived permanent additional weight gain (personal communication, Hayes, 1993). Whether this is a temporary state or a metabolic alteration is not known. This study sought to measure body weight shifts as influenced by female sex hormones. The female rat model was chosen because the controlled environment allows more accurate measures of body weight and food intake measurements than can be obtained in free living subjects keeping dietary records. The application of animal data to human physiology is often questioned. However, the rat model chosen for this study reflects many of the parameters shown in the literature to directly parallel the human female as suggested by Wade & Schneider (1992). The present study also shows a strong correlation of the rat to the human. The results of the PEPI study should relate additional information on the hormonal effects on human body weight when it is released in 1996 (The PEPI Writing Group, 1995).

RESULTS OF PHASE II: OVERVIEW

The availability of sweet and nonsweet CHO sources allowed for the additional investigation of hormonal effects. Body weight differences in hormonal treatments between Phase I and Phase II utilizing the same animals provided new information with regard to the effects of progesterone and provided evidence of regulatory mechanisms. Four food cups containing three macronutrient food choices were presented to the four OV groups. The sham rats which were used for comparison, continued with the three foods cups. The CHO source was divided into sweet and nonsweet components. The caloric content of the sweet food choice was 4 times higher in simple sugars than the nonsweet. The nonsweet carbohydrate sources were maltodextrin, cornstarch and cellulose which are classified as complex carbohydrate food choices (carbohydrate formulations Table 3.4). Protein and fat sources were the same formulation as in phase I. The sham group was not included in the following tables reported in this chapter. The tables which indicate 5 group contrasts and percentages along ANOVA factorials, and 5 group summary graphs can be found in the appendix.

BODY WEIGHT

Body weight gain in both absolute and relative measures (total and %) was greater (Table 4.2A, 5 group ANOVA p196, Figure 5.0 p212) in the OV animals than the E&P and P. Ovariectomized animals gained more body weight than hormone treated animals (Table 4.2 B total & % 4 group ANOVA contrasts 5 group ANOVA p196). P treated animals gained less than Non P (total & % 5 group p196, factorial, 197).

TABLE 4.2A PHASE II - BODY WEIGHT GAIN AND TOTAL CALORIC INTAKE

Treatment	Ovariec-tomized	OV + 17ß Estradiol	OV + 17ß Estradiol and Pro-gesterone	OV + Proges-terone	ANOVA p value
Number of Animals (n=)	10	11	12	10	
Total Weight Gain (g)	15.3±3.4 (a)	7.3 ± 2.1 (a,b)	4.6 ± 1.4 (b)	4.2 ± 2.7 (b)	p=0.009
% gain	5.3%(a)	2.8%(a,b)	1.7%(b)	1.4%(b)	p=0.013
Total Caloric Intake	953.5 ± 66.4	822.7 ± 44.8	838.1 ± 23.6	871.7 ± 39.2	p=0.188
% of total	34.1%	33.0%	31.6%	31.8%	
Caloric Conversion Ratio	15.6±3.2 (a)	7.9±2.1 (a,b)	5.5±1.7 (a,b)	4.3±3.1 (b)	p=.016

Values are means ± SE. Means in the same row with the different superscripts are significantly different from each other by Scheffé's S procedure for post-hoc comparisons

% of gain is for this 10 day period
% of total caloric intake shows total for phase

TABLE 4.2B PHASE II - GROUP CONTRASTS FOR TOTAL BODY WEIGHT GAIN AND TOTAL CALORIC INTAKE

Treatment	n	Total Weight Gain*		p value	Total Calorie Intake*		p value	Caloric Conversion Ratios	p value
				g			g		
		g	%	%	g	%	%		
OV vs H	10	15.3 ±3.4	5.3 ±1.2	p=.001	954.0 ±66.4	34.0 ±1.2	p=.045	15.6±3.2	p=.0024
	33	5.3 ±2.1	2.0 ±.4	%p=.002	843.1 ±35.9	32.2 ±.6	%p=.059	5.9±2.3	
E vs Non E	23	5.9 ±1.2	2.3 ±.5	p=.122	831.0 ±24.2	32.3 ±.7	p=.073	7.9±2.1	p=.173
	20	9.7 ±2.5	3.3 ±.8	%p=.127	913.0 ±38.7	32.9 ±.8	%p=.81	9.9±3.2	
P vs Non P	22	4.4 ±1.4	1.5 ±.5	p=.007	853.8 ±21.7	31.9 ±.6	p=.473	4.3±3.1	p=.010
	21	11.1 ±2.1	4.0 ±.7	%p=.006	884.0 ±41.1	33.4 ±.8	%p=.486	11.8±2.6	

*Mean ± SEM, α.05

Hormones = E, P, E&P
Ovariectomized = OV, no hormones
Estrogen = E, E&P
Non Estrogen = OV, P
Progesterone = P E&P
Non progesterone = OV, E

CALORIC INTAKE

There were no differences in of total caloric intake across the treatments (Table 4.2A, 4 group ANOVA factorial p196). However, in total caloric group contrasts, OV with no hormone consumed higher levels of calories than hormonally treated animals (Total Table 4.2B, 5 group p197).

CALORIC CONVERSION

Caloric conversion ratio (Figure 7.0 p214) was less in the P treated animals compared to OV animals (Total Table 4.2A). In group contrasts, OV conversion of calories into body weight was higher than in H treated animals, and P was lower than Non P (Total Table 4.2B). A progesterone effect on caloric conversion ratio was also shown with factorial analysis (4 group ANOVA factorial p197).

FAT

No main effects were observed for fat intake (total & %) among the treatment groups (Table 4.2 C). However, hormonally treated animals ate a greater percentage of fat than OV as revealed by group contrasts and E implanted rats consumed a higher % of fat than Non E (% Table 4.2D). Total calories comparing FAT, CHO, and PRO are shown in Figure 6.0 p213.

PROTEIN

Total protein consumption (Figure 6.0 p213) was higher in the OV and E&P than in P treated animals (Total & % Table 4.2C). Group contrasts also revealed that protein intake was higher with the OV than the hormonally treated animals (Total Table 4.2D). A E&P effect was shown on increased protein consumption (4 group ANOVA factorial p197).

TABLE 4.2C PHASE II - MACRONUTRIENT FOOD CHOICES IN KCAL

Treatment	Ovariec-tomized	OV + 17ß Estradiol	OV + 17ß Estradiol and Pro-gesterone	OV + Proges-terone	ANOVA p value
Number of Animals (n=)	10	11	12	10	
Total In-take of Carbo-hydrate (Calories)	524.2 ± 48.9 (a)	324.1 ± 45.4 (b)	310.4 ± 33.3 (b)	395.9±51.8 (a,b)	p=.007
% of total	56.5%	39.8%	37.0%	47.6%	p=.054
Total Intake of Protein (Calories)	78.3 ± 9.4 (a)	49.6 ± 9.5 (a,b)	85.0 ± 10.5 (a)	33.9 ± 3.0 (b)	p=.001
% of total	8.6%(a,b)	6.2%(a,b)	10.1%(a)	4.0%(b)	p=.004
Total Intake of Fat (Calories)	351.0 ± 75.7	448.0 ± 60.7	443.5 ± 37.7	441.8± 83.8	p=.683
% of total	34.9%	53.9%	53.0%	48.4%	p=.084

Values are means ± SE. Means in the same row with the different superscripts are significantly different from each other by Scheffé's S procedure for post-hoc comparisons
% of gain is for this 10 day period
% of total caloric intake shows total for phase

TABLE 4.2D PHASE II - GROUP CONTRASTS FOR TOTAL CARBOHYDRATE, PROTEIN AND FAT

Treatment	n	Carbo-hydrate		p value	Protein		p value	Fat		p value
				g			g			g
		g	%	%	g	%	%	g	%	%
OV vs	10	524.0 ±48.9	56.5 ±5.4	p=.001	78.3 ±9.4	8.6 ±1.2	p=.042	351.0 ±75.7	34.9 ±5.1	p=.228
H	33	340.9 ±43.5	41.1 ±3.0	%p=.020	57.7 ±7.7	7.0 ±.7	%p=.058	445.0 ±60.7	51.9 ±3.2	%p=.015
E vs	23	317.0 ±27.2	38.3 ±3.2	p=.003	68.0 ±7.9	8.3 ±.9	p=.216	446.0 ±34.2	53.4 ±3.6	p=.450
Non E	20	460.0 ±37.6	52.0 ±4.3	%p=.013	56.1 ±7.0	6.3 ±.8	%p=.278	397.0 ±55.9	41.7 ±4.5	%p=.042
P vs	22	349.3 ±30.4	41.7 ±3.7	p=.120	61.8 ±8.0	7.3 ±1.0	p=.616	443.0 ±42.1	50.9 ±3.9	p=.508
Non P	21	419.4 ±39.4	47.8 ±4.2	%p=.153	63.3 ±7.3	7.4 ±.9	%p=.581	402.0 ±48.1	44.9 ±4.4	%p=.418

Hormones = E, P, E&P
Ovariectomized = OV, no hormones
Estrogen = E, E&P
Non Estrogen = OV, P
Progesterone = P E&P
Non progesterone = OV, E

TABLE 4.2E PHASE II - CALORIC INTAKE OF SWEET AND NONSWEET CARBOHYDRATE

Treatment	Ovariec-tomized	OV + 17β Estradiol	OV + 17β Estradiol and Pro-gesterone	OV + Pro-gesterone	ANOVA p value
Number of Animals (n)	10	11	12	10	
SW vs. Non/SW			♦		
Sweet Carbohydrate	277 ± 26	167 ± 21	226 ± 30	164 ± 29	p=.017
%	29.2%	21.1%	26.6%	19.9%	p=.1678
Nonsweet Carbohydrate	247 ± 55 (a)	158 ± 39 (a,b)	85 ± 14 (b)	232 ±4 (a,b)	p=.015
%	27.3%	18.7%	10.3%	27.6%	p=.022

Values are means ± SE. Means in the same row with the different superscripts are significantly different from each other by Scheffé's S procedure for post-hoc comparisons.

♦ E&P intake was significantly different in SW/CHO vs. NON/SW CHO p=.001.

TABLE 4.2F PHASE II - GROUP CONTRASTS CALORIC INTAKE OF SWEET AND NONSWEET CARBOHYDRATES

Treatment	n	SW Carbo-hydrate		p value	NSW Carbohydrate		p value
		g		g			g
		SEM	%	%	g	%	%
OV vs H	10	277.2 ±25.8	29.2 ±2.5	p=.127	247.1 ±54.7	27.8 ±6.3	p=.0552
	33	185.6 ±26.7	22.6 ±3.0	%p=.120	157.9 ±30.9	18.8 ±3.6	%p=.058
E vs Non E	23	166.5 ±27.2	21.1 ±3.2	p=.253	157.5 ±39.4	18.7 ±4.4	p=.004
	20	220.7 ±27.5	24.6 ±3.2	%p=.189	202.3 ±47.2	27.5 ±5.3	%p=.006
P vs Non P	22	164.4 ±29.8	19.9 ±3.9	p=.264	231.6 ±8.0	27.6 ±4.7	p=.616
	21	221.8 ±47.1	25.7 ±2.9	%p=.301	202.3 ±94.2	22.9 ±5.4	%p=.581

Hormones = E, P, E&P
Ovariectomized = OV, no hormones
Estrogen = E, E&P
Non Estrogen = OV, P
Progesterone = P E&P
Non progesterone = OV, E

CARBOHYDRATE

Macronutrient analysis in phase II (Table 4.2 C-E Figure 6.0 p213), as in phase I, revealed that both E and E&P treatments decreased total CHO intake compared to OV (Total Table 4.2C). CHO intake was higher in the OV than in hormonally treated animals when using group contrasts (Total & % Table 4.2D). Estrogen implanted animals consumed less CHO than Non E treated animals (Total & % Table 4.2D). E combined with P had a greater effect than either separately (4 group ANOVA factorial p198).

SWEET & NONSWEET CARBOHYDRATE

Sweet vs. Nonsweet CHO was significantly different in E&P treated animals (Table 4.2E). Sweet carbohydrate consumption was significantly higher in the OV than the H treated animals in group contrasts (Total Table 4.2 F). There was an E&P effect which means that E&P combined stimulated more SW/CHO intake than E or P separately (4 group ANOVA factorial p198).

E&P treatment group had an intake of nonsweet CHO which was one third of the level consumed by the OV animals (Total & % Table 4.2E). Estrogen treatment group had a significantly lower intake of nonsweet CHO than the Non E treated animals (Table 4.2F). An E effect was observed (4 group ANOVA factorial p 198).

DISCUSSION OF PHASE II

The purpose of phase II was to continue to examine hormonal effects for another ten days on body weight, macronutrients and especially "tease out" the type of carbohydrate intake, sweet or nonsweet, as affected by exogenous female hormones. The effect of estrogen, continued as observed in Phase I, but all the other groups and especially the progesterone treated animals

adapted and did not continue to gain weight the same (Figure 17.0) as in Phase I. This is important information because currently progesterone is perceived to cause body weight gain and this can be a major reason females do not comply with HRT prescriptions given by the physician (Lerner, 1995). E&P represents the typical hormone treatment therapy prescribed by physicians. In HRT, the progesterone is added to decrease the likelihood of reproductive cancer.

When Phase II is compared to Phase I, body weight in four group analysis with both total and % analysis revealed increased body weight in the OV animals compared to P. OV gained 3 times more body weight than the P treated animals. Progesterone treated animals gained an average of only .4 g of body weight per day compared to 1.5 g in the OV treatment. Group contrasts confirmed these effects with higher body weight in OV compared to H, and Non P compared to P.

In Phase II as in Phase I, no significant differences were observed in caloric intake. The only caloric consumption differences were observed in OV animals which was significantly higher than H when group contrasts were examined. With the significant differences in body weight and the absence of significant differences in caloric intake among the treatment groups, the evidence in Phase II showing hormonal effects on caloric conversion ratios was strengthened. OV animals required fewer calories to gain a gram of body weight than did the P treated animals. When grouped, OV body weights were higher than H and Non P were higher than P. This provides evidence that hormone treatment suppresses the additional body weight gain of the OV and that specifically progesterone in Phase II suppressed body weight gain,

contrary to the results observed in Phase I. It should also be noted that the S group responded similarly to the P group in Phase II with dramatically different caloric conversion ratios contrasted to Phase I. This slowing of body weight gain may be a natural response to elevated body weight gain following surgery in Phase I.

Hormones also continued to affect food choices with significant differences in macronutrient consumption in Phase II. Macronutrient choices revealed that the percent of fat intake was higher in the H and E than in the OV and NonE treatments. This agrees with the hypothesis that FAT consumption is higher during the luteal phase. Protein intake was higher in OV and E&P compared to H and P, confirming an estrogen effect on PRO consumption. Total CHO intake was higher in the OV than in the E&P and P with an estrogen effect revealed by factorial analysis. In group contrasts OV consumed more total CHO than H and NonE consumed more total CHO than E. With regard to consumption patterns for SW/CHO, E&P had a greater effect when combined than either hormone had separately. OV consumed more SW/CHO than H. More NSW/CHO was consumed by NonE than E. Additionally, E&P consumed significantly more SW/CHO than NSW/CHO. However, the rats were clearly able to discriminate and combine both SW and NSW.

The examination of the hypothesis of hormonal effects on food intake and body weight revealed a different and unexpected result in phase II. The reason for the decrease in body weight gain in phase II in the female was further examined. One reason may be due to the regulation mechanism termed "set point", which serves as a thermostat or regulator around which upper and lower limits of body weight are maintained reported by Fantino &

Brinnel (1986). This set point regulation mechanism has also been observed in the human female (Harris, 1990).

Calories appear to be converted into body weight at a higher rate in OV animals (postmenopausal model) compared to the S animals (premenopausal model) with intact ovaries. Differences were observed in caloric intake among the five groups (Figure 6.0). Comparisons of the percentages of fat consumption of the four groups shows fat consumption to be lower in the OV compared to H and higher in the E treated animals when compared to NonE in agreement to Geiselman's et al. findings (1981). The high caloric conversion ratio of the OV group is evident in Figure 7.0. This study provides evidence that the high CHO intake of the OV group may have contributed to increased body weight gain. This information agrees with a recent report from the Center for Genetics, Nutrition, and Health in Washington, D.C., (Simopoulos, 1994) which shows that a high carbohydrate diet or conversely a low fat diet can be fattening, which also supports the research conducted by Tarttelin and Gorski (1973).

The unexplained weight gain in 25 to 30 % of the aging population (Leibel, 1995) is a subject widely discussed in the popular and professional press. Insulin resistance may provide a further explanation of increased body weight with high carbohydrate consumption in phase II (Simopolous, 1994).

Abnormalities in carbohydrate and lipid metabolism evident in hyperinsulinemia have been investigated by Reaven (1988). He has concluded that the possibility exists that resistance to insulin-stimulated glucose uptake and hyperinsulinemia are involved in the etiology and clinical course of three major related diseases; non-insulin dependent diabetes,

hypertension and coronary disease. In addition to these conditions, obesity, particularly truncal obesity, has been shown to be associated with insulin resistance and hyperinsulinemia, increased very-low density lipoprotein triglyceride, decreased high-density lipoprotein triglyceride, decreased high-density lipoprotein cholesterol and hypertension. Reaven has termed this state as "syndrome X". The current hypothesis, however, is that syndrome X and obesity, whether genetically determined or acquired, might act through insulin resistance to cause metabolic abnormalities (Reaven, 1988). From a physiological standpoint, it is important to know if insulin resistance is the first abnormality from which the others follow. Other factors that modulate insulin action should also be studied.

The increased body weight with high CHO consumption may be due to an increase in insulin resistance in the OV animals. Treatment with exogenous hormones (E and E&P) in the present study resulted in an altered rate of weight gain, variation in the intake % of calories consumed as CHO and the subsequent impact on the caloric conversion ratio. This comparison is also indicative of the important role of E and P in the above processes as it relates to macronutrient selection. It should be mentioned that this research by no means demonstrates that insulin resistance was confirmed in the OV animals or that E and P are the only hormones involved, as many other factors are changed with the ovariectomy.

Simopoulos, (1994a) recent research reveals that insulin resistance can occur in the preobese state. Typically insulin is not measured as an indication of irregular body weight gain in the human, neither was it measured as an indicator of body weight changes in this study. However, a growing

body of evidence has recently reported that with aging, adult onset diabetes is increasing and that 25 to 30 % of the population may suffer from insulin resistance (Leibel, 1995). This figure also parallels the percentage of the population known to be obese (Atkinson, 1990). Ovariectomy may affect insulin resistance and possibly the greater consumption of CHO. The concepts of metabolic parameters in obesity correlated to insulin resistance should be examined in greater detail in future research.

The environment also has an impact on insulin resistance in the form of stress, drugs, smoking, alcohol, diet and inactivity. The effects of these environmental factors have been investigated and results indicate that in the predisposed individual, saturated fats decrease insulin sensitivity and that alcohol raises blood pressure and triglycerides. Whereas regular physical exercise improves insulin sensitivity (decreases insulin resistance), reduces blood sugar and lipid levels, lowers blood pressure, reduces body fat, and leads to weight loss. Subgroups of individuals at high risk in whom markers of the predisposition exist, should be identified (Reaven, 1988) by measuring insulin resistance. Some genotypes may be more sensitive to environmental influences than others. Based on a cardiovascular disease study by Borkman et al. (1993) and assuming that dietary fatty acid intake influences the composition of muscle membrane phospholipids, the highly refined carbohydrate current food supply provides the perfect environmental influence in which genetically predisposed individuals gain excess body weight. The relationship to insulin resistance is indicated but not currently proven.

Results of the food choice and caloric regulation data in the present study may be highly relevant for the human condition. For example, low fat

cookies are perceived to be a low calorie food choice, but they truly are not low in calories. These food choices are high in calories in the form of simple sugars. With this perception and a CHO appetite an entire box of diet cookies can be consumed by a postmenopausal woman with high caloric intake and increased body weight. Even if excess calories are not consumed, a diet high in simple sugars could theoretically lead to obesity (Reaven, 1988), contrary to recent perceptions. In phase II surprisingly, a higher fat intake was observed with lower body weight in the E&P treated rats. A recent report (Allred, 1995) states "A low fat or low-calorie food is low in neither if multiple servings are consumed and excess caloric intake occurs."

In the past, cyclical variations in food intake and body weight in females, synchronous with the ovarian cycle, have been well documented. These findings are reflected in research in humans (Pliner & Flemming, 1983), in monkeys (Rosenblatt, et.al., 1980), and in numerous rodents such as the rat (Brobeck, Wheatland, & Strominger, 1947; Wade and Gray, 1979), hamster (Miceli & Fleming, 1983; Morin & Flemming, 1978) and guinea pig (Czaja, Butera, & McCaffrey, 1983; Czaja & Roy, 1975). In all these species food intake and body weight are minimal at estrus, increase, reaching a maximum during diestrus, and then decrease until the next diestrus. The dependence of these cycles on female sex hormones is attested to by their disappearance after ovariectomy and their restoration by appropriate hormonal treatment. Ovariectomy in younger female rats (Landau & Zucker, 1976; Wade, 1974), mice (Blaustein, Gentry, Roy, & Wade, 1976) and hamsters (Miceli & Fleming, 1983) increases food intake and permanent body weight increase. Carcass analyses have indicated that body weight changes represent changes in fat

mass within age and treatment groups (Galleti & Klopper, 1964; Gray & Wade, 1981; Leshner & Collier, 1973; Pallier, Aubert, & Lemonnier, 1980; Wade, 1976), total protein, and water content (Gavin, Gray, & Johnson, 1983). Data reflecting original percent body composition are needed in future research at the beginning and end of each phase.

The fluctuation in body weight observed in phase II needs further investigation. The addition to the literature of the progesterone effect on lowered body weight in the aging model is a new contribution.

RESULTS OF PHASE III: OVERVIEW

The objective in phase III was to determine hormonal effects on chocolate choices and the subsequent impact on caloric regulation and body weight beyond the observations in phase II by adding 4 caloric levels of chocolate to the existing macronutrient choices. This gave the animals 8 food cups from which to choose: the four macronutrients from phase II plus the four choices of chocolate. Chocolate choices included: high fat/ high sugar; high fat/ low sugar; low fat/ high sugar; and low fat/ low sugar. Hormonal effects on chocolate choices and macronutrient preferences were observed. The weight gain was not significantly different in this phase among OV, E, P, and E&P which received the chocolate food choices however body weight was significantly different from S which received the 3 macronutrient choices throughout the entire study. The addition of another group, S receiving all food choices, would have allowed the investigation of surgery vs. food choices but due to death rates early in the study, this group was eliminated.. Statistical analysis of the 4 groups compared to 5 groups yielded different results.

An interesting finding of phase III was that caloric intake was chosen from the nutrient deficient chocolate choice to the exclusion of the more nutrient dense FAT, CHO, and PRO food cups, which were complete diet choices that included all the macronutrients in the recommended amounts. Results also revealed that the sweet/nonsweet carbohydrate food choices were ignored in preference to the chocolate flavored choices. Caloric intake from macronutrients FAT, PRO, and CHO was calculated both separately and with those found in the chocolate food cups for comparative analysis.

BODY WEIGHT

No significant differences were observed in total body weight gain when excluding the S groups for the statistical analysis (Table 4.3A). The S group continued to gain weight at a rate similar to phase II, while not being given the chocolate choices (5 group % S vs H p199). There were no group contrast differences in body weight (Table 4.3B). Body weight gain increased across all treatments in phase III compared to phase II for ovariectomized rats regardless of the type of hormone replacement. Weight gain among the treatment groups in this phase was not significantly different. Four group ANOVA is shown in the tables in the text. Five group analyses, percentages, and factorials appear in the appendix. Body weight was significantly different when S was included (Total & % 5 group ANOVA p 199).

TOTAL CALORIC INTAKE

No significant differences were observed in total caloric intake among treatments as affected by exogenous female sex hormones (Total Table 4.3A). There were no total contrast differences in caloric intake (Table 4.3B). Total

TABLE 4.3A PHASE III - BODY WEIGHT GAIN AND TOTAL CALORIC INTAKE

Treatment	Ovariec-tomized	OV + 17ß Estradiol	OV + 17ß Estradiol and Pro-gesteron e	OV + Proges-terone	ANOVA p value
Number of Animals(n)	10	11	12	10	
Total Weight Gain (g)	21.7 ± 2.6	26.2 ± 3.1	25.3 ± 2.7	25.9 ± 3.5	p=.708
% gain	7.1%	9.6%	8.8%	8.5%	p=.351
Total Caloric Intake	942± 56	898±30	977± 44	988± 57	p=.516
% of total	33.7%	36.0%	36.8%	36.0%	
Caloric Conversion Ratio	22.7±2.0	29.2±3.2	25.3±2.1	26.2±3.7	p=.450

Values are means ± SE. Means in the same row with the different superscripts are significantly different from each other by Scheffé's S procedure for post-hoc comparisons
% of gain is for this 10 day period
% of total caloric intake shows total for phase (including chocolate)

TABLE 4.3B PHASE III - GROUP CONTRASTS FOR BODY WEIGHT GAIN AND TOTAL CALORIC INTAKE

Treatment	n	Body Weight Gain*		p value	Total Calorie Intake*		p value
				g			g
		g	%	%	kcal	%	%
OV vs	10	21.7 ±2.6	7.1 ±.7	p=.251	942.1 ±56.0	33.8 ±1.1	p=.815
H	33	25.8 ±3.1	9.0 ±.6	%p=.268	955.0 ±25.0	36.4 ±.6	%p=.794
E vs	23	25.8 ±2.0	9.2 ±.7	p=.515	940.0 ±27.6	36.6 ±.6	p=.578
Non E	20	23.8 ±2.2	7.8 ±.7	%p=.526	965.2 ±39.4	34.9 ±.9	%p=.457
P vs	22	25.6 ±2.1	8.6 ±.7	p=.577	983.4 ±34.3	36.5 ±.8	p=.184
Non P	21	24.0 ±2.1	8.4 ±.7	%p=.563	919.1 ±30.5	35.0 ±.7	%p=.145

*Mean ± SEM, α.05

Hormone = E, P, E&P
Ovariectomized = OV, no hormone
Estrogen = E, E&P
Non Estrogen = OV, P
Progesterone = P, E&P
Non progesterone = OV, E

calories reflect intake from all sources. Caloric intake was significantly different when S was included (Total & 5 group ANOVA p 199).

CALORIC CONVERSION RATIO

No significant differences were observed among OV treated animals, with or without exogenous hormone replacement treatment (Total Table 4.3A). Caloric conversion ratios were not significantly different when S was included.

TOTAL FAT

No significant differences were observed in % or total fat among the groups (Table 4.3C). There were no group contrast differences in fat intake (Table 4.3D). However, it should be noted that high fat, high sugar chocolate intake contributed the major portion of high fat intake in the caloric consumption profile (Total & % Table 4.3E). No significant differences were observed in 5 group analysis.

TOTAL PROTEIN

No significant differences were observed in protein intake (Table 4.3C). Factorial analysis revealed an interaction effect of E&P which means that with regard to protein intake, the presence of E with P had a greater effect than either alone (4 group ANOVA factorial p200).

TOTAL CARBOHYDRATE

For the first time in the thirty day study there were no significant differences in total CHO intake for both total and % (Table 4.3C). There were no differences in CHO in group contrasts analysis (Table 4.3D). In this phase of the study neither the estrogen nor progesterone treatments affected the carbohydrate consumption. No significant differences were observed in N/SW CHO or in SW/CHO.

TABLE 4.3C PHASE III - MACRONUTRIENT FOOD CHOICES AND CHOCOLATE

Treatment	Ovariec-tomized	OV + 17β Estradiol	OV +17β Estradiol and Pro-gesterone	OV + Pro-gesterone	ANOVA p value
Number of Animals (n)	10	11	12	10	
Intake of Carbohy-drates (Calories)	432.7 ±37.0	441.9 ±38.1	389.7 ±23.8	431.9 ±58.9	p=.814
% of total	46.8%	43.8%	45.3%	44.1%	p=.965
Intake of Protein (Calories)	72.1 ±16.9	34.2 ±7.7	74.2 ±10.8	49.2 ±11.5	p=.062
% of total	7.5%	4.0%	8.1%	4.8%	p=.087
Intake of Fat (Calories)	437.3 ±51.2	474.3 ±59.1	463.2 ±37.5	507.0 ±72.8	p=.858
% of total	45.7%	52.1%	46.6%	51.1%	p=.708

Values are means ± SE.
% of gain is for this 10 day period
% of total caloric intake shows total for phase (includes chocolate)

TABLE 4.3D PHASE III - GROUP CONTRASTS FOR CARBOHYDRATE, PROTEIN AND FAT

Treatment	n	CHO Intake kcal		p Value	PRO Intake kcal		p Value	Fat Intake kcal		p Value
				g			g			g
		g	%	%	g	%	%	g	%	%
OV	10	432.7	46.8	p=.819	72.1	7.5	p=.174	437.3	45.7	p=.612
vs		±37.7	±4.2		±16.9	±1.7		±51.2	±4.3	
H	33	420.0	44.5	%p=.716	53.3	5.7	%p=.153	480.2	49.8	%p=.596
		±42.9	±2.5		±10.0	±.7		±56.5	±2.7	
E	23	416.9	44.6	p=.698	54.2	6.2	p=.592	468.5	49.2	p=.994
vs		±25.3	±2.6		±7.8	±1.0		±35.6	±2.9	
Non E	20	432.3	45.5	%p=.647	60.6	6.1	%p=.479	472.2	48.4	%p=.972
		±34.0	±3.4		±10.3	±1.0		±44.1	±3.6	
P	22	437.4	44.8	p=.547	62.2	6.6	p=.477	483.1	48.6	p=.698
vs		±28.9	±2.7		±8.2	±1.0		±38.2	±2.9	
Non P	21	410.2	45.2	%p=.562	53.1	5.7	%p=.424	456.7	49.1	%p=.629
		±29.8	±3.3		±19.7	±1.0		±38.6	±3.6	

*Mean ± SEM, α.05

Hormone = E, P, E&P
Ovariectomized = OV, no hormone
Estrogen = E, E&P
Non E = OV, P
Progesterone = P, E&P
Non progesterone = OV, E

TABLE 4.3E PHASE III - MACRONUTRIENT FOOD CHOICES CONTRASTS OF CHOCOLATE CHOICES*

Treatment	Ovariec-tomized	OV + 17β Estradiol	OV + 17β Estradiol + Proges-terone	OV + Proges-terone	p value
High Fat/ High Sugar Choco-late cal.	330.0 ± 65.4 (59g)	271.9 ± 38.3 (48.9g)	400.4 ± 41.8 (71.9g)	346.3 ± 49.1 (62.2g)	p=.302
%	33.9±5.5	31.3 ±5.2	40.8±3.9	34.7±3.4	p=.483
High Fat/ Low Sugar Chocolate cal.	32.1 ± 8.7 (a,b) (6.2g)	13.9 ±3.9 (a) (2.7g)	53.3±12.1 (b) (10.3g)	41.3± 10.9 (a,b) (8.0g)	p=.036
%	3.2±0.7	1.5±.4	5.3±1.1	4.3±1.1	p=.024
Low Fat/ High Sugar Chocolate cal.	54.9 ± 21.4 (20.7g)	42.2 ± 8.5 (15.9g)	46.6 ± 12.1 (17.6g)	33.7 ± 10.0 (12.7g)	p=.757
%	6.2±2.6	4.6±0.9	4.6±1.1	3.7±1.1	p=.729
Low Fat/ Low Sugar Chocolate cal.	22.8 ± 4.7 (19.2g)	27.1 ±5.3 (22.8g)	20.7 ± 2.8 (17.4g)	27.0 ± 5.5 (22.7g)	p=.757
%	2.6±0.6	3.0±0.5	2.2±0.3	3.0±0.7	p=.675

*All chocolate combined:

Total kcal	439.8.	355.1	521.0	448.4
% Total kcal	46.7%	39.5%	53.3%	45.4%

Values are means ± SE. Means in the same row with the different superscripts are significantly different from each other by Scheffé's S procedure for post-hoc comparisons

% of gain is for this 10 day period

% of total caloric intake shows total for phase (chocolate only)

TOTAL CHOCOLATE

All groups that were given chocolate demonstrated a preference for High Fat/ High sugar chocolate, consuming from 272 to 400 kcal of this food choice. All other chocolate choices were ingested in smaller amounts. E&P consumed higher levels of HF/LS than E (Total & % Table 4.3E). P consumed higher levels of the HF/LS than the Non P (Total & %). The effect of P was a greater than E or E&P on HF/LS chocolate intake (4 group ANOVA factorial p200). During this phase, 40 - 53% of calories consumed came from the chocolate food choices.

DISCUSSION OF PHASE III

The overriding effect of chocolate on food choice, body weight gain and caloric regulation in Phase III was a new contribution in this research. The paste form was similar to the consistency of bonbons. Much higher levels of chocolate consumption than previously expected were recorded. Careful observation of the data revealed that the rats increased their preference for the chocolate food cups across all treatment groups, and did so to the exclusion of the more macronutrient dense selections, FAT, CHO and PRO. These three macronutrient food choices had been carefully planned and balanced to provide all essential vitamins and minerals but were dramatically excluded in preference to chocolate. The S group did not receive the chocolate choices but served as a comparison group of endogenous hormonal effects on FAT, CHO, and PRO in the normally cycling rat of the same age. When the S group was included for comparison, the following significant differences were observed: body weight, caloric intake, and caloric conversion ratio differences. Therefore, not surprisingly, in group contrasts, S was significantly different

than H. Figures 8.0 through 10.0 (pp 215-217) give the reader a review of the five group comparisons. The tables and analyses in this section for Phase III reflect four group comparisons in total and percent. Body weight, caloric intake, and caloric conversion ratios in the four groups were not significantly different.

Macronutrient choices as influenced by hormones were evident but no differences were observed in the SW/NSW CHO profile in the analyses in phase III. Factorial analysis provided additional information with regard to the E&P effect of protein consumption. This indicates that the combination of hormones has a more powerful influence on protein consumption than when estrogen and progesterone are administered separately. To parallel the evidence from the current study with the human female; estrus compares to the follicular phase when estrogen levels are endogenously higher and diestrus compares to the luteal phase when progesterone levels are elevated. The reader will recall that E&P is typically prescribed for women with a uterus and E alone to those who have had a hysterectomy.

The question: do hormones affect chocolate consumption was clearly answered. The overriding effect of HF/HS chocolate on macronutrient choices in the OV retired breeder female rat was quite evident. The suppressing effect of estrogen on body weight in Phase I and the suppressing effect of progesterone on body weight in phase II was "wiped out" in phase III when chocolate was presented as a food choice. The only significant differences in chocolate consumption in phase III were the preferences for HF/LS in the E&P vs E treatments and the group contrasts in P vs. NonP. In this case, the fat in the chocolate (HF/LS) seemed to be a greater attraction than the sucrose to

the E&P group. When E and P effects (for HF/LS) were compared in factorial analysis the P effect was greater than either the E&P combined or the E alone. In a broader sense, this may indicate that females on a continuous combined regimen of estrogen (Premarin®) and progesterone (Provera®) may experience a greater attraction to chocolate, regardless of sweet flavor sources. And that when P levels are higher (in the luteal phase or when cyclical hormone replacement is prescribed, E for 12 days and E&P for 12 days), FAT and chocolate are more important than sucrose. Chocolate and fat may be craved (in contrast to sugar) when both E and P are elevated (in diestrus in the rat) (luteal phase in the cycling female) and when progesterone is taken in hormone replacement therapy.

This indicates that the progesterone effect relates more to fat choice than to sucrose preference. This progesterone effect was further emphasized in group analysis in both total and percent. P treated animals preferred the HF/LS in significantly greater quantities than Non P treated animals. This shows that the rat can discriminate between sweet flavors when they are combined with chocolate and FAT. This potential parallel to the human adds merit to the use of the Sprague-Dawley rat in this type of research. The human literature strongly reports the progesterone effect of chocolate and FAT choices in the luteal phase when progesterone levels are high. This may help explain the hypothesis of preferred (craved) fat and chocolate intake just prior to menstruation.

The increase in total chocolate calories emphasizes the attraction to chocolate compared to the more nutrient dense foods. They were clearly ignored in preference to chocolate. It is presumed that the S group would

also have chosen chocolate had the choice been given and that chocolate would also have increased the S body weight. Further research should be conducted to determine if the surgical effect of the removal of the ovaries affected these food choices or altered metabolic conversion rates.

This evidence challenges the Bowen and Grunberg (1990) hypothesis which reported increased sweet preference in the luteal phase with documented progesterone levels. However, the reader will recall that their sweet choices also included fat and chocolate. This evidence agrees with the earlier work by Abraham (1984) who reported cravings for chocolate in the luteal phase. Pliner and Fleming (1983) also found that both body weight and food intake were significantly higher during the luteal phase than during the follicular phase in menstruating women. In a later study (1983) they also reported decreased sucrose preference both before and after a sucrose load during the luteal phase but not in the follicular phase.

Aaron's work (1975) which supports these findings reported greater pleasantness of the sweet taste in the follicular phase which lowered during the luteal phase when progesterone levels were higher and estradiol and estrone were lower. Without regard for the hormonal phase, Smith and Sauder (1969) stated that 85% of those who craved chocolates also included themselves in the group that craved sweets. The current data clearly indicate that eating behavior research should always reveal the stage in the menstrual cycle or source of exogenous hormones when reporting food intake data in the future.

These results also emphasize the importance of studying not only sugar preference but also fat preference. When the evidence from the three phases

is combined, the "teasing out" of the sweet, fat and chocolate components are possible. In phase I , H (E and P) consumed a significantly higher % of dietary FAT than OV. And P treated animals consumed significantly more total FAT than NonP. Evidence from phase II clearly indicates a sweet preference (3 times higher) in the E&P treated animals over nonsweet carbohydrate. And in Phase III, the study shows that the high fat/low sugar chocolate is preferred by the E&P animals in contrast to E. This indicates that in the aging female rat, FAT and chocolate may be preferentially chosen over the sweet when both estrogen and progesterone levels are elevated. This also provides evidence of the value of using the same rats over the 30 day study under the influence of the same hormones with three different food choice patterns. Through varying the macronutrients in phase I we were able to discern hormonal effects on the choice of FAT, CHO or PRO. In phase II we were able to further discriminate between the sweet and nonsweet. Then in phase III we were able to separate the hormonal effects further with the four caloric levels of chocolate by separating high and low fat levels with saccharin and sucrose and observing the P effect of FAT and chocolate over sucrose.

Flavor, palatability and the opioid effect of chocolate are likely contributing factors (Drenowski, 1985). Data on the preferred HF/HS type of chocolate were also observed (Giraudo, et al., 1993). The rats chose the more palatable chocolate choices as is typically seen in the human population. No definitive evidence in animals or humans was reported to explain the preference for chocolate. Discriminatory palatability differences in the Sprague-Dawley rat have been shown in earlier taste tests in rats (Hamilton, et al., 1995). Addition research is needed to investigate the excessively high

levels (40-53% during the 10 day study) of chocolate intake in all ovariectomized animals.

This paste form of chocolate in four caloric levels may be a new addition to the eating behavior taste research arena. The paste form used was quite palatable, easy to manage, and the most acceptable form of chocolate chosen by rats in a pilot study when chocolate chips, chocolate bars, chocolate syrup and the paste form were compared.

No female rat studies comparing chocolate consumption under hormonal influence have been reported. However, earlier studies measuring other parameters have reported the use of chocolate in the Sprague-Dawley rat (Runyan et al, 1990, Richard et al, 1982). In one such study, cocoa powder was fed to male and female Sprague-Dawley rats in a multi-generational study to examine toxicity and carcinogenicity (likelihood of cancer). No evidence of carcinogenicity was reported in either gender (Tarka, et al., 1991).

Few recent human chocolate eating behavior studies have been conducted which measure hormonal influences. One human study measuring fat, carbohydrate and chocolate indicated increasing preferences for chocolate as body weight increased and obesity advanced over time (Drewnowski, et al., 1985). Increased body weight was positively correlated to food preferences for fats, sweets and chocolate. In another study, Hetherington & Macdiarmid (1995) measured responses to three different presentations of chocolate in two groups of females: overeaters and moderate eaters. Three conditions of presentation were (a) a fixed amount of milk chocolate; (b) ad lib access to milk chocolate; and (c) a self-selected amount of the individual's most preferred form of chocolate. The results revealed that variables associated

with the excitation of appetite were higher in overeaters and variables associated with the inhibition of appetite were lower in the overeaters relative to the controls. This study suggests that investigations of individuals who eat certain foods to excess can help define normative and aberrant eating behavior. This is an important contribution in eating behavior research. However, actual levels of chocolate intake were not reported. The measurement of hormonal profiles in future female studies should be documented and correlated to food intake parameters.

Interestingly, in the present study with chocolate consumption in phase III, rats increased caloric intake compared to phase II, and no significant differences were observed in body weight gain and total caloric intake within this 10 day period in the four groups receiving chocolate choices. Also, no differences were observed in overall FAT, CHO, and PRO intake which included the fat and carbohydrate from the chocolate choices. The actual intake of the nutrient rich FAT, CHO, and PRO food choices was decreased as fat and carbohydrate were consumed from the HF/HS chocolate choice.

Choices from the chocolate food cups which were consumed in large quantities (40-53% of caloric intake) were nutrient deficient. This means that the rats were not consuming a nutritionally balanced diet. With respect to micronutrients, they were getting about one-half the daily NRC recommendations (Reeves et al, 1993). While this is not critical for a ten day study period, on a long term basis the animals may become seriously deficient, depending on individual requirements. Aging human females may also choose nutrient deficient snack foods to the exclusion of more nutrient

dense foods that could help combat osteoporosis, B-vitamin deficiencies and other nutrient related diseases.

Often in the human, the additional chocolate is eaten for dessert. To date we do not have conclusive evidence in the aging female as to whether this is added as excess caloric intake or is chosen as the major portion of the diet in preference to nutrient dense foods as was observed with the female rat. However, the rats did increase total caloric intake by 9.24% from phase II to phase III when the caloric preference included chocolate. Both the rat and the human female may choose chocolate in preference to more nutrient dense foods when the choice in available with subsequent body weight increase and negative health benefits.

RESULTS: ACROSS ALL PHASES

The following results are reported for both five and four group analysis. Data in tables are based on 5 group ANOVA. All percentages, contrasts and factorials are in the appendixes. There are limitations in the interpretation of these data across all phases. One weakness is that the same animals were used throughout the 3 phases in the 30 day study. Ideally animals would have been sacrificed at the conclusion of each phase.

BODY WEIGHT

Five Group Analysis: Total body weight was higher in the OV and P animals compared to the S over the 3 phases in both total and %(Table 4.4A).Group contrasts revealed that in averages over the 30 days, S was lower than H, OV was higher than H, E was lower than Non E (Total & % Table 4.4B).

Four Group Analysis: The total body weight was higher in the OV animals compared to H and E was significantly lower than Non E (4 group

Total & % ANOVA contrasts p 201). An estrogen effect was observed (4 group ANOVA factorial p202) which means that E had a greater effect on decreased body weight than E&P or P alone.

TOTAL CALORIES

Five Group Analysis: The average in total caloric intake over the three phases for S was lower than the averages for E, E&P, P, and OV (Total & % Table 4.4A). S was lower than H (total) and E revealed a trend of a lower average caloric intake than Non E in group contrasts (Table 4.4B).

Four Group Analysis: ANOVA showed no significant differences in the caloric intake across the phases (4 group ANOVA).

CALORIC CONVERSION RATIO

Five Group Analysis: S was lower than P and OV (Total & % Table 4.4 A). S was lower than H, and E was lower than Non E (Total Table 4.4B) in group contrasts. An effect was observed in factorial analysis (p203).

Four Group Analysis: A trend, P,.058, was observed in 4 groups (p202). E was lower than Non E (Total 4 group ANOVA p202).

TOTAL FAT

Five Group Analysis: Total fat intake was not significantly different (Table 4.4C). OV was lower in percentage (not total) of fat consumed than H across all phases (Table 4.4D) (Figure 14 & 16 p221-223).

Four Group Analysis: No differences were observed.

TOTAL PROTEIN

Five Group Analysis: S and P consumed less protein than E&P (Total & % Table 4.4C). S consumed less protein than H (Total Table 4.4D).

TABLE 4.4A TOTAL STUDY - BODY WEIGHT GAIN AND TOTAL CALORIC INTAKE AND CONVERSION RATIOS

Treatment	Sham	Ovariec-tomized	OV + 17β Estradiol	OV + 17β Estradiol and Pro-gesterone	OV + Pro-gesterone	ANOVA p value
Number of Ani-mals (n)	12	10	11	12	10	
Total Weight Gain (g)	27.3± 4.4 (a)	61.4 ± 6.5 (b)	39.9± 5.2 (a,b)	44.6 ± 3.2 (a,b)	57.7 ± 6.0 (b)	p=.001
% gain	10% (a)	23% (b)	16%(a,b)	16.7%(a,b)	21% (b)	p=.001
Total Caloric Intake (kcal)	2184±69 (a)	2794±144 (b)	2493 ±83 (b)	2650 ± 93 (b)	2743±100 (b)	p=.001
Conversion Ratio •	12.2 ± 1.8 (a)	22.1 ± 2.8 (b)	16.0±.2.0 (a,b)	16.8 ±1.1 (a,b)	21.0 ± 2.0 (b)	p=.003

Values are means ± SE. Means in the same row with the different superscripts are significantly different from each other by Scheffé's S procedure for post-hoc comparisons

• Conversion ratio is body weight increase divided by total caloric intake over 30 days x 1000 or g wt. gained/1000 kcal consumed

TABLE 4.4B TOTAL STUDY - GROUP CONTRASTS OF BODY WEIGHT GAIN AND TOTAL CALORIC INTAKE

Treat	n	Total Weight Gain*		p value	Total Calorie Intake*	p value	Conversion Ratio	p value
				g	kcal			
		g	%	%	Mean±SEM		Mean±SEM	
S vs	12	27.3 ±4.4	10.4 ±1.8	p=.001	2184 ± 70	p= .002	12.2±1.8	p=.008
H	33	47.0 ±4.8	17.6 ±1.1	%p=.001	2627 ± 47		17.8±2.0	
Ov vs	10	61.4 ±6.5	23.1 ±2.4	p=.024	2794 ± 144	p= .165	22.1±2.3	p=.063
H	33	47.0 ±4.8	17.6 ±1.1	%p=.024	2627 ± 47		17.8±2.0	
E vs	23	42.3 ±2.8	16.2 ±1.2	p=.001	2582 ± 61	p= .053	16.3±1.0	p=.008
No E	20	60.5 ±4.4	21.9 ±1.6	%p=.005	2769 ± 90		21.9±1.5	
P vs	22	50.6 ±3.4	18.6 ±1.2	p=.917	2692 ± 67	p= .599	18.7±1.1	p=.920
No P	21	50.1 ±4.7	19.2 ±1.8	%p=.998	2636 ± 86		18.9±1.6	

*Mean ± SEM, α.05

Sham = S
Hormones = E, P, E&P
Ovariectomized = OV, no hormones
Estrogen = E, E&P
No Estrogen = OV, P
Progesterone = P E&P
No progesterone = OV, E

TABLE 4.4C TOTAL STUDY - MACRONUTRIENT FOOD CHOICES IN KCAL

Treatment	Sham	Ovariec-tomized	OV + 17β Estradiol	OV + 17β Estradiol and Pro-gesterone	OV + Proges-terone	ANOVA p value
Number of Animals (n)	12	10	11	12	10	
Intake of Carbohy-drates (Calories)	811 ±90 (a)	1499± 112 (b)	1086± 117 (a,b)	1067± 65 (a,b)	1241±131 (a,b)	p=.001
% of total	38.0%	53.9%	43.6%	40.4%	46.1%	p=.082
Intake of Protein (Calories)	118±17 (a)	235 ± 31 (a,b)	172 ± 30 (a,b)	291 ± 30 (b)	149 ± 28 (a)	p=.001
% of total	5.5%(b)	8.6%(a,b)	7.1%(a,b)	11.2%(a)	5.4%(b)	p=.003
Intake of Fat (Calories)	1255±137	1060± 130	1236±142	1291±101	1352±197	p=.701
% of total	56.5%	37.5%	49.3%	48.3%	48.5%	p=.088

Values are means ± SE. Means in the same row with the different superscripts are significantly different from each other by Scheffé's S procedure for post-hoc comparisons

TABLE 4.4D GROUP CONTRASTS OF TOTAL STUDY MACRONUTRIENT FOOD CHOICES INTAKE

Tmt	n	Intake of Carbohydrate *		p value	Intake of Protein*		p value	Intake of Fat*		p value
				total			total			total
		total	%	%	total	%	%	total	%	%
S	12	811	38.0	p=.008	118	5.5	p=.007	1255	56.5	p=.812
vs		±90	±4.7		±17	±.8		±137	±5.1	
H	33	1126	43.2	%p=.057	208	8.1	%p=.061	1291	48.7	%p=.961
		±104	±2.2		±29	±.8		±147	±2.5	
OV	10	1499	53.9	p=.004	235	8.6	p=.352	1060	37.5	p=.176
vs		±112	±3.5		±31	±1.2		±130	±3.8	
H	33	1126	43.2	%p=.036	208	8.1	%p=.396	1291	48.7	%p=.046
		±104	±2.2		±29	±.8		±147	±2.5	
E	23	1057	42.0	p=.007	227	9.3	p=.164	1298	48.8	p=.692
vs		±64	±2.4		±24	±1.1		±87	±2.8	
Non E	20	1410	50.0	p=.051	199	7.0	%p=.194	1161	43.0	%p=.716
		±84	±3.1		±23	±.8		±117	±3.6	
P	22	1146	43.0	p=.192	227	8.6	p=.541	1319	48.4	p=.232
vs		±70	±2.7		±27	±1.1		±102	±3.0	
NonP	21	1283	48.5	%p=.212	202	7.8	%p=.612	1152	43.7	%p=.295
		±92	±2.9		±22	±.9		±96	±3.3	

*Mean ± SEM, α.05

Sham = S
Hormones = E, P, E&P
Ovariectomized = OV, no hormones
Estrogen = E, E&P
Non Estrogen = OV, P
Progesterone = P, E&P
Non progesterone = OV, E

Four Group Analysis: E&P animals consumed more protein than both E and P treatment groups (Total p203). An E&P effect was observed (4 group ANOVA factorial p 203). This means that the presence of estrogen with progesterone had a more significant impact on protein consumption than estrogen or progesterone alone.

TOTAL CARBOHYDRATE

Five Group Analysis: Carbohydrate intake was lower in S than OV (Total Table 4.4C). S consumed less than H (total), OV consumed more than H (total & %), E consumed less than Non E (total), (Table 4.4D).

Four Group Analysis: OV was higher than H, and E was lower than Non E (Total 4 group ANOVA p203). An estrogen effect was shown on carbohydrate intake (4 group ANOVA factorial p204). This means that the presence of estrogen alone had a more significant impact on lowered CHO intake than P or E&P.

POST MORTEM ANALYSIS

TOTAL BODY COMPOSITION

Total body composition was compared using a percentage basis to correct for the larger and smaller rats (Table 4.4E). No significant differences for the percentage of body fat were observed by the ether extraction method. No differences in percentage of moisture were determined in measures of carcass homogenate taken in a drying oven (Table 4.4E). Neither were there percentage differences observed in total body composition from protein. (Table 4.4E).

CHOLESTEROL

Levels of serum cholesterol in the S treatment were lower (Table 4.4F) than the H treated animals (5 group ANOVA group contrasts p226).

HDL CHOLESTEROL

Additionally levels of serum HDL Cholesterol were lower (Table 4.4F) in S than H (5 group ANOVA group contrasts p228).

CHOLESTEROL-HDL RATIO

No significant differences in total cholesterol to HDL ratios were observed among treatments (p225-226).

SERUM ESTROGEN

No significant differences in serum estrogen levels were observed (Table 4.4G).

SERUM PROGESTERONE

A significant difference was observed in serum progesterone (Total Table 4.4G, and 4 group ANOVA p205).

SERUM TESTOSTERONE

No significant differences were observed in 5 group serum testosterone levels (Table 4.4G, 4 group ANOVA p205). Group contrasts revealed differences in 4 groups analysis. P vs. NonP revealed significant differences in testosterone levels (Total 4 group ANOVA p205).

WEIGHT OF HEART

No significant differences in heart weight were observed among the treatments (Table 4.4H).

TABLE 4.4E TOTAL STUDY - BODY COMPOSITION

Treatment	Sham	Ovariec-tomized	OV + 17β Estradiol	OV + 17β Estradiol and Pro-gesterone	OV + Proges-terone	ANOVA p value
Number of Animals(n)	12	8	10	11	11	
Fat (%)	8.21± .37	8.22 ± .72	9.10 ± .78	10.0± .48	8.95 ± .39	p=.141
Protein (%)	9.77 ± .15	9.29 ± .25	10.07±.71	9.3 ± .23	9.00 ± .19	p=.240
Water (%)	80.30±.52	81.30±.58	79.60±1.42	79.20±.63	80.40±.22	p=.466

Values are means ± SE.

TABLE 4.4F TOTAL STUDY - CHOLESTEROL AND HIGH DENSITY LIPOPROTEIN CHOLESTEROL

Treatment	Sham	Ovariec-tomized	OV + 17β Estradiol	OV + 17β Estradiol and Pro-gesteron e	OV + Pro-gesteron e	ANOV A p value
Number of Animals (n)	12	10	11	12	10	
Cholesterol mg/dl	82.3 ± 5.1	105 ± 5.6	102.0 ± 7.6	110.0 ± 7.9	101.0 ±11.7	p=.116
Group Contrasts	(a) ∇	(b)*	(b)*	(b)*	(b)*	p=.014
HDL Cholesterol mg/dl	71.8 ± 4.5	80.8 ± 5.0	87.7 ± 7.7	93.6 ± 7.6	84.4 +9.0	p=.216
Group Contrasts	a) ∇	(b)*	(b)*	(b)*	(b)*	p=.034

Values are means ± SE. Means in the same row with the different superscripts are significantly different from each other by Scheffé's S procedure for post-hoc comparisons.
∇ * Denotes group contrasts.
Group means: CHOL: S: (82.3±5.1), OV (104.5±8.2)
HDL-C: S: (71.8±4.5), OV (86.6±7.3)

TABLE 4.4G HORMONE LEVELS BY RADIOAMMUNOASSAY

Treatment	Sham	Ovariec-tomized	OV + 17ß Estradiol	OV + 17ß Estradiol plus Pro-gesterone	OV + Proges-terone	ANOVA p value
Number of Animals (n)	12	11	10	11	11	
Estradiol	141.0 ± 46.6	79.0 ± 24.7	142.0 ± 21.2	140.0 ± 18.6	109.0 ± 29.6	p=.521
Progesterone	10.1 ± 2.2	4.9 ± 1.7	4.2 ±.9	11.0 ± 2.8	18.7 ± 6.3	p=.033
Testosterone	.97 ± .46	.08 ± .08	.37 ± .29	.05 ± .03	.65 ± .32	p=.139
Group contrasts	(a,b)	(b)	(b)	(a)	(a)	p=.050

Values are means ± SE. Means in the same row with the different superscripts are significantly different from each other by Scheffé's S procedure for post-hoc comparisons

WEIGHT OF LIVER

S had lower liver weights than E&P and P (Total Table 4.4H). S and OV also had lower liver weights than H (Table 4.4I).

WEIGHT OF UTERUS

Five Group Analysis: OV and P were lower than E&P, E, and S (Total Table 4.4H). S was higher than H, OV was lower than H, E (total Table 4.4I p204).

Four Group Analysis: Both OV and P were lower than E&P and E (Total p233). OV uterine weights were lower than H and E was higher than Non E (Total p206). An estrogen effect on uterine weights was observed (4 group ANOVA factorial p206).

FAT PERCENTAGE OF LIVER

Five Group Analysis: Percentage fat (% Table 4.4J) in the E group was lower than Non E.

Four Group Analysis: An E effect was observed in liver fat content (% 4 group ANOVA factorial, p207).

WEIGHT TO LENGTH RATIO

Five Group Analysis: E treated animals had a lower (Total Table 4.4 K) weight to length ratio than Non E.

Four Group Analysis: An E effect was observed in weight to length ratios (4 group ANOVA factorial, p 207).

DISCUSSION OF THE TOTAL STUDY

These data for the total study across all phases indicate that exogenous female sex hormones affect caloric conversion ratios in retired breeder female rats given novel food choices. And that over time the rats treated with

TABLE 4.4H TOTAL STUDY - WEIGHT OF ORGANS

Treatment	Sham	Ovariecto-mized	OV + 17ß Estradiol	OV + 17ß Estradiol and Progester-one	OV + Progester-one	ANOVA p Value
Number of Animals (n)	12	10	11	12	10	
Weight of Uterus (g)	.74 ± .05	.24 ± .03	.62 ± .05	.61 ± .05	.40 ± .04	p=.001
Weight of Heart (g)	1.17 ± .04	1.27 ± .07	1.2 ± .03	1.21 ± .07	1.29 ± .05	p=.458
Weight of Liver (g)	9.15 ± .23	9.85 ±.19	10.40 ±.32	10.50 ±.24	10.60 ±.37	p=.002

Values are means ± SE. Means in the same row with different superscripts are significantly different from each other by Scheffé's S procedure for post-hoc comparisons.

TABLE 4.4l TOTAL STUDY GROUP CONTRASTS FOR WEIGHT OF ORGANS

Treat-ment	n	Weight of Uterus	p value	Weight of Heart	p value	Weight of Liver	p value	Weight of Bone	p value
		Mean ± SEM		Mean ± SEM		Mean ± SEM		Mean ± SEM	
S vs	12	.738 ± .049	p=.001	1.17 ± .04	p=.254	9.15 ± .23	p=.001	1.19 ± .05	p=.355
H	33	.545 ± .046		1.23 ± .05		10.50 ± .31		1.14 ± .04	
OV vs	10	.242 ± .026	p=.001	1.27 ± .07	p=.625	9.85 ± .186	p=.052	1.17 ± .05	p=.589
H	33	.545 ± .046		1.23 ± .05		10.50 ± .31		1.14 ± .04	
E vs	23	.616 ± .047	p=.001	1.21 ± .06	p=.174	10.50 ± .28	p=.488	1.13 ± .04	p=.431
Non E	20	.322 ± .035		1.28 ± .06		10.20 ± .28		1.17 ± .05	
P vs	22	.508 ± .046	p=.091	1.25 ± .06	p=.713	10.60 ± .31	p=.096	1.17 ± .05	p=.483
Non P	21	.430 ± .037		1.24 ± .05		10.10 ± .25		1.13 ± .05	

Sham = S
Hormones = E, P, E&P
Ovariectomized = OV, no hormones
Estrogen = E, E&P
No Estrogen = OV, P
Progesterone = P E&P
No progesterone = OV, E

TABLE 4.4J % FAT CONTENT OF THE LIVERS

Treatment	Sham	Ovariec-tomized	OV + 17ß Estradiol	OV + 17ß Estradiol and Pro-gesteron e	OV + Proges-terone	ANOVA p value
Number of Animals (n)	12	10	10	11	12	
Fat (%)	1.4±.2	1.9 ± .3	1.3 ± .2	1.2 ± .1	2.1 ± .4	p=.086
Group contrasts	(a,b)	(a)	(b)	(b)	(a)	p=.010

Values are means ± SE. Means in the same row with the different superscripts are significantly different from each other by Scheffé's S procedure for post-hoc comparisons. It should also be noted that factorial analysis shows significant effects of estrogen at (p=.02).

Group means: E: (1.3± .5), lower than NonE (2.0 ± .4) in group contrasts.

TABLE 4.4K TOTAL STUDY - WEIGHT TO LENGTH RATIOS

Treatment	Sham	Ovariec-tomized	OV + 17β Estradiol	OV + 17β Estradiol and Pro-gesterone	OV + Proges-terone	ANOVA p value
Number of Animals (n)	12	10	11	12	10	
Weight to Length Ratio g/cm Δ	16.7 ± .4	18.3 ± .5	16.8 ±.4	17.2 ± .4	18.3 ± .4	p=.015
Group Contrasts	(a,b)	(a)	(b)	(b)	(a)	p=.004

Values are means ± SE. Means in the same row with the different superscripts are significantly different from each other by Scheffé's S procedure for post-hoc comparisons

Δ Weight to length ratio is length in centimeters divided by weight in grams

Group means: E: (17±.4) lower than NonE (18.3±.5).

exogenous female sex hormones regulate body weight. Evidence of this regulation phenomonen is the caloric conversion ratio for phase II which was lower than for phase I and phase III. In order to examine these parameters separately in an aging model over time the combined effects of the 30 day study also seem worthy of consideration. Eating behavior literature typically reflects this type of data collection. Therefore, the following report relates the statistical analysis of all three phases combined.

The carryover effect from phase to phase of the body weight parameter and subsequent effects on body composition should be noted. The effect can be moderated by considering the % weight gain and growth rates in the animals. In future studies, the collection of body weight data from the day the hormones were implanted is recommended. Significant body weight differences may also be due to growth rates (Figure 17.0) and interactions with exogenous hormones. A recent study addressing body weight changes, indicates that children had significantly greater percentages in body weight changes than their adult parents, which indicates that caloric conversion ratio differences can be age related (Epstein et. al., 1995). Growth hormone which has recently been approved by FDA for human use in this country could be measured in future studies. The future measurement of insulin is also recommended. The observations within each phase have already been presented. The results at the end of the study are a summary of the hormonal effects for the 30 day period (Figures 1.0-17.0 pp 208-224).

The estrogen effect on body weight as observed by Wade & Schneider (1992) was found only by factorial analysis when the results were combined over the 30 day study. When Sham is removed for four group analysis E is

significantly lower than the OV treatment. Body weight was higher in the OV and P treatments in both total and percent when compared with the sham in five group analysis. As previously stated, this may reflect chocolate choices not allowed by the S group and E effects on suppressed body weight. Group contrasts with five groups reveal H to be significantly higher than S, And in both four and five group analysis OV was higher than H and Non E higher than E. The application to hormonal effects on body weight in the human forms a nice parallel. The endogenous estrogen pool representative of the premenopausal female appears to keep body weight at lower levels. Once surgical or natural menopause occurs, the natural endogenous source in removed. Exogenous estrogen appears to keep body weight gain lower compared to the increased body weight gain in the OV female with no exogenous supplemental hormonal therapy.

The more interesting concept in the aging model is the evidence that under hormonal influence, calories are converted into body weight at varying degrees as shown in significant differences in caloric conversion ratios. The higher the caloric conversion ratio, the fewer kcal are required to gain a gram of body weight. Calories in this case are more efficiently converted. Both OV and P conversion ratios were significantly higher than S. Factorial analysis also revealed an estrogen effect on lower caloric conversion ratios. Group contrasts confirmed the aging human model implications; ovariectomized animals given hormone supplements had significantly higher caloric conversion ratios than S. Non E conversion ratios were higher than E. Estrogen would appear to suppress caloric conversion into body weight in both the ovariectomized and normally cycling females.

Macronutrient choices were significantly different but not in ways which were expected. An interesting new concept was that hormone treated animals chose higher percentage levels of FAT than OV with significantly lower body weights. PRO was chosen by the E&P group in significantly higher levels than the S and P which was confirmed by factorial analysis. Group contrasts identified higher levels of PRO intake in H treated animals compared to S. This may be of value if the PRO intake is chosen in the form of red meat in the bleeding postmenopausal female who may be deficient in heme iron.

CHO was consumed in greater quantities in the OV animals compared to S. In this study higher levels of CHO in the OV animals resulted in significantly higher body weight gain. Estrogen had a greater influence alone than P or combined E&P in suppressing CHO intake. When grouped, with regard to CHO consumption, OV was significantly higher than H, Non E higher than E and H was higher than S. The hormonally untreated postmenopausal female model may consume higher quantities of highly refined carbohydrate snack foods, convert calories into body weight more efficiently and ultimately gain more body weight than those who receive exogenous female sex hormones.

The evidence of hormonal effects on body weight gain, macronutrient food choices and caloric regulation was observed early in the study, when estrogen implanted animals gained significantly less body weight than the progesterone and OV animals. Further, the additional body weight gain effects of progesterone were of short duration, which may occur at the onset on menopause or immediately following surgical removal of the ovaries. Progesterone effects of increased fat and chocolate intake are often observed

in the younger female in the luteal phase followed by periods of fasting during the menses or follicular phase (Dalvitt-McPhillips, 1983). This adds strength to the argument that the Sprague-Dawley female rat is a good model to study in examining human pharmaceutical and physiological questions. Earlier studies in the intact female rat have shown greater caloric intake and body weight in the diestrus phase when progesterone levels are elevated (Gray & Greenwood, 1983). These data also agree with the estrogen effect observed in younger rats in earlier studies which reflects decreased body weight gain and the trend to lower caloric intake in estrus when estrogen is elevated (Wade, 1975, 1976). The more meaningful interpretation of these physiological endocrine parameters to the aging female may be that the declining estrogen pool seen during this period of life (Wing, 1992) may contribute to metabolic alterations leading to the additional body weight observed in the aging rat model in this study.

The rats in this study may have adapted as evidenced by reduced body weight possibly via a setpoint mechanism over time. In phase II, the second ten day period, the rate of gain decreased for S, E&P and P when compared to the first 10 days of the study for phase I. Body weight increased again in phase III when chocolate was available. In this study, higher CHO both as a total and percentage of calories consumed was identified with ovariectomy and increased caloric conversion to body weight gain. Increased fat intake both as a total and percentage of calories consumed was observed in treatment groups with decreased body weight gain and lower caloric conversion ratios. These concepts are clearly contradictory to current hypotheses that low fat diets produce decreased body weight gain. The

evidence shown by the changes in caloric conversion ratios could indicate physiological and metabolic mechanisms which should be investigated in future research.

Before these conclusions can be considered for potential theoretical change, the potential weaknesses which could have affected in these results should be discussed. Inclusion of the sham group in statistical analyses must be addressed because the more palatable novel food choices were never offered to this group. The sham group was only offered the three nutrient dense food choices FAT, CHO and PRO throughout the study. This group served as a comparison and represented the normally cycling female. Future studies should include a sham group which received all food choices. This was the original design of the study, but due to the high death rate following the two surgeries early in the study, one group was unfortunately eliminated. Therefore the same three macronutrient food choices ingested throughout the study could have affected the body weight data. There is a strong likelihood that the sham group did not gain at the same rate due to the difference in food choices. The other obvious factor is the difference in surgical processes which left the ovaries in tact in the sham animals. Sham body weight was less than other treatments in all other phases except for phase I when all groups received the same food choices. They did not receive the same SW/NSW CHO and chocolate options in phases II and III, respectively, as the other groups. This design and information may skew the results when the sham is compared to the other treatment groups. The other four groups of ovariectomized animals who had access to the more palatable choices showed clear preferences when the options were available in phase III. This

has marked implications for examining behavioral food choice mechanisms. To extrapolate this evidence to the human model may indicate that when more palatable food choices are available, these may be chosen to the exclusion of other well balanced, nutrient dense foods that may not be as delicious but are nourishing and essential to good health. This concept agrees with the work of Rolls (1993) in the study of sensory specific satiety.

Caloric intake from the three macronutrient choices was significantly different in group contrasts between the sham and the hormone treated animals with a trend toward significance when estrogen and nonestrogen were compared. No caloric intake differences were shown when only the four treatment groups were individually compared. When macronutrient food choices were observed and compared, carbohydrate, protein and fat consumption were shown to be influenced by the hormone treatments. CHO was preferred by OV. Protein choice was almost twice as high for E&P compared to the P, and a progesterone treatment effect on FAT consumption during the 30 day study period.

In the total study exogenous hormones were clear indicators of body weight differences between S and OV, with regard to caloric intake. Caloric conversion for S was lower than the for OV and P groups. Two additional parameters that possibly played a role in this study which should be measured in future research are; the hormonal effects on increased voluntary exercise and hormonally induced lethargy.

Due to the biases potentially created by the sham receiving the three food choices throughout the study, the four OV groups should be discussed. When the four OV groups were compared, E treatment resulted in lower caloric

conversion of food intake into body weight than in the Non E treated animals. Differences of caloric conversion ratio and body weight to length ratio both revealed a trend between the OV and hormonally treated rats. The differences in weight to length ratios may relate to the rate of growth in the rat model. Exogenous female sex hormones may alter length growth rates in rats. Exogenous hormone treatments may increase involuntary activity, voluntary exercise, or metabolic differences and should be examined in future research. These effects separately or combined may have influenced the data used to calculate the rate at which calories are converted into body weight. Obviously, in the four OV groups, ovariectomy results in the absence of endogenous estrogen influence. Exogenous estrogen prevented the increased body weight which was observed in the OV, P and S (endogenous) rats in phase I in this study and in the OV and P in the total study (Galletti & Klopper, 1964; Gray & Greenwood, 1984). Tracking the effects over longer periods of time in the aging model may provide additional information.

At the conclusion of the study, animals were sacrificed and the post mortem results were analyzed. The following could have affected the statistical outcomes and should be mentioned. NonE treated animals were significantly longer at the conclusion of the study than the E treated animals which could affect body weight data. Liver weights were significantly higher in the E&P and P treated animals than S indicating the need for further analysis into the hepatic markers related to exogenous female sex hormones.

The significantly different uterine weights confirm the bioactivity of the exogenous hormone implants. This important marker was shown in significantly different uterine weights with atrophy of the uterus clearly seen in

the OV group. E and E&P implants were equally effective in approaching maintenance of pre-ovariectomy uterine weights. P alone could only partially maintain uterine weight. Thus estrogen was the most critical hormone in preventing atrophy of the uterus in the ovariectomized rat. The additional biomarker used to assess the activity of the implants was radioimmunoassay of the serum to measure progesterone, estradiol and testosterone. Serum progesterone levels were significantly higher in the animals implanted with progesterone. Testosterone levels were higher in animals with progesterone implants. Estradiol levels were not found to be significantly different. Biological evidence of uterine weights clearly reveals that estradiol levels were also different, however a more sensitive form of RIA analysis should be used in the future to measure this hormone.

Surprisingly, body fat distribution did not vary across the treatments. In fact, total body composition (%) analysis revealed no significant differences in fat, water and protein among the five treatment groups. Mean body composition data, obtained upon sacrifice, showed 9% of the total weight as fat, 11% as protein, 80 % as body water. The rats consumed almost half of their caloric intake from fat, but deposited only 9% of it in body fat stores as shown in total body composition analysis. This information is contrary to earlier reports by Wade and Gray (1979) in which adiposity was measured in terms of lipoprotein lipase activity and correlated to increases in body fat storage. A potential weakness in the use of lipoprotein lipase as a marker of changes on body fat composition is that lipoprotein lipase activity may increase proportionately as body weight increases and may not necessarily reflect differences in % body composition. Earlier studies reported lipoprotein lipase

increased with increased body weight in the absence of measures for protein and water which were not measured in proportional body composition. Therefore the body fat composition (%) reported in Wade's earlier studies may not be different and therefore flawed in reporting. If increases in body fat as a percent of total body composition were reported by Wade, the results might present a different picture. Similar results to those found in the present study might be shown. Preliminary and concluding body composition data by DEXA should be measured in future studies but were not possible in the present study. The methods of measuring total and % body fat increases should be examined.

Body composition analysis was questioned by Clark and Tarttelin (1982). They report conflicting results from chemical analysis for example, comparing intact rats, to the weight of body fat in OV rats as a percentage of body weight. To examine the differences, Sprague-Dawley rats were ovariectomized at three ages: 3 days, 4 weeks and 7 weeks and were subsequently sacrificed at four ages, 7, 9, 12 and 15 weeks for chemical analysis of carcass and skin. Chemical compositions were analyzed at % wet weight and as component weights by two-way analysis of variance. Ovariectomy increased overall body weight without causing obesity. The weight gain of the OV rat was mainly due to true growth response. OV treatment increased fat reserves but slowed the growth of other body components including the skeleton. E treatment reversed this process. Clearly body composition data must be examined with the same age, species and treatment to provide comparable data. Earlier studies have reported increased (Leshner & Collier, 1973; De Smet, 1953), decreased (Galleti &

Kloper, 1964, Rebuffe-Scrive, 1987), and unchanged body fat end points (Bogart, Lasley & Mayer, 1944; Holt, Keeton, and Vennesland, 1936; Nyda, deMajo, & Lewis, 1948; Reed, Anderson, & Mendel, 1932). Data in the postbreeder female which has been ovariectomized and implanted with female sex hormones were not evident in the literature.

Body composition can also be influenced by the availability of calorically consistent food choices. It should also be noted that in earlier studies (Wade & Gray, 1979; Wade & Schneider, 1992) animals were not offered choices of macronutrients or food choices of sweet, nonsweet and chocolate. The food choices in their studies consisted of lab chow and sucrose in water. Body weights were different and therefore body composition in the younger rat could also have been affected. The present study indicates that the aging female model changes in body weight but that the ratios in total body composition analysis of fat to protein and body fluids remain consistent across the treatments.

Total study caloric conversion ratios of food intake to body weight were shown to be lower in the sham than the other treatment groups (Figure 12.0). The effect of estrogen was first seen in phase I in the E group. These animals gained less weight per calorie than the other hormonal treatments. This evidence indicates that the OV and P implanted animals convert calories into body weight at a higher efficiency rate than the S and E treatment groups (Figure 12.0). The significance shown in the sham group is clearly influenced by the phase III data in which the S group was not given chocolate choices. There appeared to be differences in body weight, although calorie levels were not significantly different. These differences can also be described as the ratio

at which feed is converted to body weight. The caloric conversion ratios provide evidence that calories are converted into body weight at differing rates possibly due to changes in basal metabolic rate, hormonally induced lethargy or activity levels which were not measured in the present study. This concept requires further investigation. The data leading to caloric conversion ratios reported in this paper on the postbreeder female rat is not consistent with evidence reported by Wade and Schneider (1992) in a review of gonadal influence on body weight and adiposity. Their previously reported data from younger rats implanted with female sex hormones, indicated that the research contained in this report would constitute a valuable contribution in the body of knowledge with regard to the aging female rat model (Wade, personal communication, 1995). An earlier study (Roy & Wade, 1977) adds strength to the findings of the current study, food intake had to be restricted to 80% of the norm to keep weight stable in the estrogen deficient rats. This suggests that these animals may have had similar caloric conversion ratios to the animals in this research.

The current information suggests that when comparing research data on different age animal models (e.g. neonatal vs 4 months vs 10 months), the age and reproductive status may reflect different metabolic implications which affect eating behavior and body weight. Similar results may be seen in studies with other aging animal models. In the human, Wing (1992) has reported that additional weight gain occurs as a result of aging. The term "aging" encompasses many biological alterations. Most parameters measured in younger rats compared to the postbreeder rat reveals a different profile. Wing's suggestions in the human female may be true in the aging female rat.

The question of declining estrogen as correlated to body weight gain during the life span may become clearer when tests for estrogen are less expensive and more commonly used. Currently LH and FSH are measured as indicators of the menopausal state. Additional research should be conducted to measure body composition changes as body weight increases are observed in the aging process. Additionally, the percent body weight gain shown as adipose tissue compared to muscle tissue (% lean body mass) in the female taking hormone replacement needs further investigation.

In review, the impact of hormonal differences on the caloric intake and body weight gain for the total study can be viewed in Figure 11, the subsequent resulting caloric conversion ratios in Figure 12. Figure 13 indicates different patterns of body weight gain over the three phases. Figure 14.0 reveals influence of hormonal treatments on the percent of total caloric intake from FAT, CHO, and PRO. The higher total and % of fat intake is most obvious in this chart. Total kcal intake from each of the three phases is shown in Figure 15.0. Total caloric intake from all sources in Figure 16.0 clearly demonstrates the overwhelming effect of chocolate in phase III. Figure 17 allows the reader to view a line graph of body weight over the 30 day study. Phase II begins on day 11 and Phase III in day 21.

In future studies the measurement of pre and post markers of total body composition (% fat, protein and water) would be of great value in measuring effects of exogenous female sex hormones. Differences have been observed in the total body weight between treatments with no significant differences in % body composition. Differences have also observed hormonal effects in macronutrient choices and different caloric conversion ratios. Total dietary fat

was high across all treatments (37.5 - 56.5%). Across all phases the OV group (which consumed the lowest percentage of calories from fat) gained the most BW. The total percentage of fat in body composition analysis was S 8.2%, OV 8.22%, E 9.46%, E&P 10%, P 8.64% (p=.0945). Therefore, the higher percentage of fat intake from macronutrient food choice was not converted proportionately into percentage of body fat stores.

Therefore the extensive body of research and current reports in the popular press which indicate that the lowering of fat intake will result in decreased body weight are not in evidence from this research. This "low fat concept" is so widespread that the higher fat intake as seen in the sham treatment group with lower body weight and the lower fat intake seen in the ovariectomized animals with the greatest weight gain could easily have been thrown out as false data. However, this contribution to the literature is meaningful and should be discussed. The possibilities which explain this phenomenon may include: (a) fat is a satiating macronutrient and therefore suppresses the desire to consume additional food, (b) gut motility of foods containing higher levels of fat decreases the rate of digestion and provides a longer period of absorption and assimilation, (c) high fat foods may create a pleasure factor in eating behavior parameters that overrides other factors not met by low fat food items, and, (d) the biochemistry of appetite through systemic messenger signals whether hepatic or neural or otherwise, may be more or less efficient under the influence of certain hormones.

There is an extensive body of literature on the effects of estrogen on feeding behavior, but the relevance of these studies to the control of energy balance has yet to be fully established. According to Wade and Schneider

(1992) there is a complete absence of any unifying consensus as to how estrogen modulates feeding behavior and body weight, although several hypotheses have been proposed. One hypothesis tested is that estrogens produce anorexia by directly affecting the mechanisms that normally terminate meals. It has been suggested that estrogen promotes the effectiveness of various peripheral short-term satiety signals by trigeminal (Beretter & Barker, 1976) pancreatic and/or gastrointestinal action (Bailey & Matty, 1972; Blaustein & Wade, 1977; Lichtenberger, Nance, & Gorski, 1976; Nance, 1983; Wade & Gray , 1979), or direct action on the metabolism of adipocytes (Wade & Gray, 1979). The current study is in direct opposition to these reports because there were clearly body weight differences when ANOVA of caloric intake revealed no significant differences.

Additionally, numerous arguments speak against Wade and Schneider's (1992) hypothesis (Geary, et al, 1994; Nance, 1983; Ramirez, 1980). Other authors have suggested that the effects of estrogen on feeding behavior result from a hormone-dependent shift in the long-term control of the energy balance, which fixes the level of regulated body weight or fat stores. Redick et al. (1973) were the first to suggest that estradiol does not suppress food intake directly, but dictates a low level of body weight gain. Such a mechanism would correspond to a shift-down of the setpoint of body weight regulation and, as a consequence, feeding would be secondarily suppressed in animals which have excess weight as a regulatory attempt to readjust body weight to a lower regulated level. While this hypothesis has been repeatedly evaluated (Landau & Zucker, 1976; Nance, 1983; Fantino & Brinnel, 1986; Leibel, et al, 1995) and strengthened by numerous experimental arguments, it

has never been directly verified. Indeed the very nature of body weight setpoint is not known (Mrosovsky & Powley, 1977; Toates, 1983; Wade & Zucker, 1970a), so hormone-induced variations have never been directly appreciated.

Additionally, the elegantly designed method of evaluating the body weight setpoint in rats through food hoarding behavior during a period of reduced body weight (Fantino & Brinnel, 1986) provides strong evidence that physiological mechanisms are strongly in place. In the wild, hoarding behavior is a specific response in rodents, occurring mainly in autumn, and this response is useful in improving their long-term energy balance (Anderson & Krebs, 1978; Launay, 1975). Fantino and Brinnel (1986) reported that in female rats as well as in male, the mass of food hoarded during extended three-hour experimental sessions increases when body weight decreases. Fantino measured body weight, food intake and food hoarding behavior in female rats fed ad lib and correlated these measures with the ovarian cycle. All three parameters fluctuated synchronously with the 4 day cycle with estrus (two days prior to ovulation) and diestrus (two days following ovulation). But he detected that the critical level of body weight for the onset of hoarding behavior was 13.2 g lower at estrus than at diestrus. His research provides direct evidence in the rat of the estrogen effect of fluctuation of the body weight setpoint with the ovarian cycle under hormonal influence.

The question of additional body weight gain as related to total body composition in this model is of interest. The absence of percent change in body fat stores may provide new information in the aging rat model and in the future, help to explain shifts in body fat disposition in the human. Women may

comment, "I weigh the same at 50 as I did at 30, but my clothes don't fit the same way." Percentage of fat to lean muscle tissue and percent moisture may actually not be different as shown in the present experiment. Shifts in waist to hip ratios, and upper body and lower body weight may occur (Haarbo, et al., 1991). The shifts in location of body fat stores may be perceived as body weight gain if measures are obtained by triceps skin folds in the upper body in the aging model (Folsom, et al., 1989). These measures should not be compared to the younger model to reflect body weight gain. Shifts to upper body adiposity and waist to hip measurements are evident in the aging human female (Brezezinski & Wurtman, 1993; Armellini, et al., 1990; Peiris, et al., 1989). Methods of measuring the validity of these parameters are currently under evaluation but have not yet been approved by FDA for human use in the medical setting (Troisi, et al., 1995). Evaluation and measurement of body weight and body composition changes over time will be included in future human research.

Exogenous female sex hormones are among the highest selling pharmaceutical agents in the industry (Wallis, 1995). Postmenopausal females are currently choosing hormone replacement therapy to combat the negative symptoms of menopause and as a protective therapy against osteoporosis and cardiovascular disease. This is the reason that the two most prevalent, estrogen (17 β estradiol) and progesterone were chosen for this study to examine subsequent physiological effects (Richard, 1986; Butera & Beikirich, 1989). The estrogen group was chosen to reflect the follicular phase in the normally cycling female, estrogen replacement therapy in the female who has had a hysterectomy or the estrogen phase in hormone replacement

therapy. The estrogen and progesterone group was chosen to reflect current hormone replacement therapy and mid cycle hormonal profiles in the normally cycling female. And the progesterone treatment group was observed to show exclusive effects of progesterone. Progesterone is often prescribed to combat uncontrolled vaginal bleeding, prevent miscarriages, or as hormone replacement for women whose genetic patterns reflect a likelihood for reproductive cancer (Notelovitz & Tonnessen, 1993). Dosages and reasons physicians prescribe exogenous female sex hormones vary.

Body weight and food intake are major concerns during this period in the life of a female. Often the comment is heard, "I am eating the same amount but gaining additional weight." This perception has not been addressed in depth by the medical community but has initiated major monetary expenditures ($33 billion, [Atkinson, 1990] previously cited) in the population. The obese are not the only ones who invest in weight loss programs. The investment to lose an extra 10 to 20 pounds of additional body weight is an expense incurred by a large segment of the weight conscious population. The body weight fluctuations which result in either permanent or temporary body weight gain may be affected by female sex hormones as shown in this study. Low fat food items are expensive and not effective in achieving the desired result (Allred, 1995). When excess calories are consumed in the form of simple sugars, these simple sugars may initiate additional caloric ingestion. (Geiselman, et al., 1981; Simopoulos, 1994b). The data observed in the current study (Figures 13 and 14) agrees with reports by Rolls (1992): that caloric intake is more important in body weight gain than fat grams consumed.

In earlier research on rats, a high rate of weight gain seen in the ovariectomy has been prevented or reversed by injection of estradiol (Landau & Zucker, 1976; Wade & Zucker, 1970b) or by unilateral implants of estradiol benzoate in the ventromedial hypothalamus (Nunez, Gray, & Wade 1980; Wade & Gray, 1979). The present study supports the information in these reports. In several younger species, endogenous as well as exogenous estrogens have revealed a suppressive effect on both body weight gain and food intake (Czaja, Butera, & McCaffrey, 1983) which is not reflected in the current study. The earlier reports and the current results can explain why body weight is minimal at estrus, when circulating estradiol is maximal (Czaja, Butera, & McCaffrey, 1983; Morin & Fleming, 1978) but does not explain the non significant food intake results and subsequent differences in caloric conversion ratios.

Accordingly, the simplistic concept which has been reported in weight loss programs that the ingestion of 3,500 additional calories will produce a pound of body weight gain or that the deprivation of 3,500 calories will cause a pound of decrease in body weight may not be true in all cases. There may be metabolic effects of aging during the menopausal years which can be directly correlated to the decline in endogenous female sex hormones such as estrogen with subsequent variations in the way calories are converted into body weight. This research shows that in the aging female rat, the absence of endogenous estrogen creates the condition whereby calories are converted into body weight at a higher ratio.

Body weight has become an important consideration in the quality of life in the female. Genetics and lifestyle changes currently seen in the population

may contribute to differences observed in the human. Undefined levels of hormonal therapy designed to relieve the symptoms of menopause (hot flashes, vaginal dryness, night sweats) may create problems. Currently, laboratory documentation of endogenous levels prior to the prescribing of exogenous levels is not the normal clinical procedure. Perhaps this assessment should be considered in the future in symptomatic individuals before the decision is made on whether to prescribe 10 mg. or 2.5 mg of Provera early in menopause or immediately following surgery when body weight seems to be affected most by progesterone (eg. Phase I). In addition dosages of exogenous female sex hormones may need to be individually determined through laboratory analysis in the future. Currently, women are prescribed levels of exogenous female sex hormones in dosages which apply to the larger population and often based on patterns established in Europe. This protocol may not be appropriate for all women. Perhaps individual analysis and cultural differences should be considered in future research. Current discomforts and body weight gain may be dose related and affect compliance problems (OB/GYN physicians, personal communication, 1995). Recent reports reveal that the earlier indicated 8% lifetime compliance rate may be declining.

The findings reported in this document in female postbreeder rats may provide preliminary data to design research studies in the human. Clearly, the human model is the best model for the study of the human. Human female caloric conversion ratios should be examined in postmenopausal females taking various sources and dosages of hormone replacement therapy. There are clearly body weight and caloric fluctuations in the ovariectomized post

breeder rat. There may also be differences in the postmenopausal human female. Exogenous female sex hormones are probably here to stay, as they improve the quality of life in the postmenopausal female. More information is needed, and additional research should be conducted.

The fluctuations shown in body weights observed in phase I and II of this research reveal increased body weight gain with subsequent decrease in the rate of gain as was reported by Fantino & Brinnel (1986). The complex molecular mechanisms by which discrete ingestive behavior, continuous energy expenditure, and dynamic energy storage in adipose tissue are integrated remain unknown. The demonstration that "leptin" (Haalas, et. al., 1995) can reduce food intake and body weight in obese and lean mice provides further evidence for the hypothesis that a circulating protein-based signal, generated in adipose tissue, acts on control neuronal networks and plays an important role in the regulation of feeding behavior and energy balance. The failure to achieve these results in other genetic strains is consistent with the hypothesis that a genetic defect in some renders them unable to appropriately respond. Perhaps this may occur as a result of a defect in the receptor or postreceptor signaling pathway (Campfield, et. al., 1995). Several lines of evidence argue for circulating signals that act in the brain to regulate feeding behavior and energy balance. Future research to examine aging and gender differences will be needed to interface exogenous female sex hormones and the affects of leptin. The altered caloric conversion ratios in the present study may be evidence that female sex hormones play a role and should be included in future investigations.

CHAPTER 5: SUMMARY CONCLUSIONS & IMPLICATIONS

The focus of this research was the body weight implications of the relationship between macronutrient food choices and exogenous female sex hormones. The body weight consequences of changes in physiological status through the ovariectomy with and without hormone replacement were examined. Changes in macronutrient preference, caloric conversion ratios, and additionally the measurement of body composition and post mortem parameters were also examined. Contempory models of the regulation of energy balance investigate and emphasize the physiological signals that control energy intake. These results reflect the outcome of those signals.

The female rat model was chosen to obtain data on body weight, caloric regulation, and macronutrient choices in a controlled environment. This sequence of studies using the postbreeder rat model (to examine a postmenopausal and normally cycling female) provided an arena in which to examine questions that could otherwise not be investigated and answered in free living human subjects. Following is a summary of the conclusions from the present study:

PHASE I:

1. Exogenous estrogen in ovariectomized rats caused a significant decrease in body weight gain without significant differences in caloric intake (there was a trend toward lower caloric intake in the estrogen treated rats).
2. Caloric conversion ratios (grams of body weight gain divided by caloric intake x 1000) revealed an estrogen effect and were lower for H vs. OV (total) and E vs. Non E (total) among the groups.

3. Exogenous female sex hormones affect macronutrient choices with statistical analysis as group contrasts: (a) Higher levels of FAT (total) were consumed by P vs. NonP treated animals (confirmed by factorial analysis) and H vs. OV (%), (b) Higher levels of PRO were consumed by H vs. S (total & %) and E vs. Non E (total & %), (c) Higher levels of CHO were consumed by OV vs. H (total & %), Non E vs. E (total & %) and Non P vs. P (total & %) (all CHO results confirmed by factorial).

PHASE II:

1. There may be time related metabolic differences in the conversion of calories into body weight after the ovariectomy. Decreased body weight gain in phase II compared to phase I for the S and P groups may indicate set point (age, gender) adjustments over time.
2. Caloric conversion ratios were lower for H than for OV and P vs. Non P. The physiological effects of ovariectomy without supplemental hormones may produce a carbohydrate appetite as demonstrated by increased caloric intake from both sweet and nonsweet carbohydrate. E&P chose 3 times more SW than NSW.
3. Exogenous female sex hormones affect macronutrient choices with statistical analysis as group contrasts: (a) More FAT was consumed by H vs. Non H (%), and E vs. Non E (%), (b) More PRO was consumed by OV vs. H (total & %), (c) More CHO was consumed by OV vs. H (total & %), Non E vs. E (total & %, factorial E effect), (d) More SW/CHO was consumed by OV vs. H (total), (e) More

NSW/CHO was consumed by Non E vs. E (total & %) (also factorial E effect).

PHASE III

1. The chocolate novel food choices in phase III produced a higher rate of body weight gain. Access to chocolate eliminated both the reduced rate of weight gain caused by exogenous estrogen in Phase I and Phase II and the set point adjustment or adaptation with subsequent reduced conversion of calories into body weight in the progesterone group observed in phase II.
2. Caloric intake was significantly lower (total) in the Sham (which did not receive chocolate) than the OV treatments (caloric conversion ratios were not significantly different in 5 group compared to 4 group analysis). A sham group receiving chocolate should be included in future research.
3. Chocolate (a choice only in phase III) was consumed at 40% to 53% of total caloric intake in ovariectomized animals with or without HRT to the exclusion of the nutrient dense macronutrient food choices. P vs. NonP preferred higher levels of HF/LS chocolate which indicates that in the luteal phase chocolate and FAT may be chosen in preference to sucrose.

TOTAL STUDY:

1. Exogenous estrogen prevented the increased body weight which was observed in the OV, P and S rats in phase I in this study and in the OV and P in the total study. Overall body weight gain for the 30 days was greatest in animals with no estrogen, the OV and P

treatments. The P group (as well as the S) adjusted in phase II with lower rate of body weight gain equal to that of the E group. In Phase III, the S group did not receive chocolate while the P group increased body weight gain with chocolate as a choice. Body weight gain was higher according to group contrasts in H than S (total & %), OV was higher than H (total & %) and Non E was higher than E (total & %). Ovariectomy without hormone replacement was shown to be the greatest indicator in increased body weight. Therefore in menopause or with surgical removal of the ovaries, HRT is indicated for suppression of increased body weight gain.

2. An estrogen effect was observed for caloric conversion ratios. Caloric conversion ratios were lower for S vs. H (total) and E vs. NonE (total). Total caloric intake was higher in the OV treatments when compared to the S (total) with a trend toward lower caloric intake with E.
3. In the aging female, palatable chocolate choices may be preferred over a nutritious balanced diet. E&P preferred SW over NSW/CHO. Exogenous female sex hormones according to group contrasts affected macronutrient choices: (a) More FAT was consumed by H vs. OV (%), (b) More PRO was consumed by H vs. S (total), (c) More CHO was consumed by H vs. S (total), OV vs. H (total &%), and NonE vs. E (total).
4. The OV rats consuming the highest caloric levels of carbohydrates (total & %) gained the most body weight. Of the OV treatments, those

receiving hormones (H) consumed the highest levels of fat (%) and gained the least amount of body weight (total & %).

5. Typical regimes of hormone replacement (E or E&P) therapy do not produce increased body weight gain in the aging female rat. Hormonal effects on changes in caloric conversion ratios could indicate physiological and metabolic mechanisms which should be investigated in future research.

POST MORTEM:

1. Variations in percent of fat intake (40% to 60%) did not result in treatment differences in body composition. Mean body composition was 80% body water, 10 % fat, 10 % protein across all groups at the conclusion of the 30 day study.
2. Cholesterol and HDL cholesterol levels were lower (total) in the normally cycling rats (S) which produce endogenous hormones as opposed to the OV rats implanted with exogenous female sex hormones.
3. Uterine weights confirmed estrogen bioactivity and serum levels of progesterone verified the release of progesterone from the implants. Livers of the H treated animals weighed significantly more than S (total) or OV (total) and the (percent) fat content of the livers of the E treated animals was significantly higher than Non E. This may indicate a metabolic or hepatic influence on all of the results.

The caloric conversion ratios observed in this study may project future research results in the human female taking hormone replacement therapy. The body weight fluctuations in the first two phases may also reflect the

metabolic implications of the ovariectomy and the effort by the body to stabilize at a set-point. This physiological metabolic alteration may cdcbe a temporary weight gain that will stabilize in the human as observed in rats in phase II compared to phase I of the present study. In the human, the perimenopausal period (often 1 to 3 years) following the initial decline in estrogen may be the most critical period regarding body weight gain.

Historically it has been assumed that fat is associated with satiety and weight gain. However, the story may be different for humans under the influence of hormonal fluctuations. The role of carbohydrates (chow vs. balanced macronutrient food cups) in the study of appetite and hunger has received relatively little attention in the animal feeding literature. It has been assumed that all carbohydrates are satiating (Geiselman, et al., 1981). However, Geiselman and her colleagues (1987) have shown that carbohydrates do not always produce satiety. Instead, carbohydrates (simple sugars) appear to stimulate appetite and physiological hunger. This information is of particular significance in this research. The group that gained the most body weight also consumed the highest levels of both sweet and non/sweet carbohydrates. Fat intake was found to be elevated in the groups implanted with exogenous female sex hormones that gained less body weight.

The information from the present study should be accompanied by the following warnings. If novel food choices containing sweets, fats and chocolates are presented immediately following ovariectomy or natural menopause, body weight may be altered and conversion ratios of food intake into body weight may also be increased. This research shows that timing and age with the free choice for chocolate may be a major risk factor. The animals

chose chocolate to the exclusion of other balanced macronutrient food choices in the diet. Poor nutrition in menopause may confound hormonal effects and add additional body weight. This body weight increase in the past may have been blamed on the hormones, when in fact, poor food choices may have been the culprit. These observations may go unnoticed in the aging human population. Taste responsivity reported by Rodin et al. (1976) and others is known to play a major role in food choice. The novel flavor and the opioid effect of chocolate may contribute to increased intake and resulting body weight gain (Drewnowski et al., 1985).

A state of malnutrition can develop over time if this preference dominates to the exclusion of more nutritious foods. The aging female who lives alone may prefer the convenience and taste of sweet, fat and chocolate snack foods over more nutritious food choices that may be more labor intense to prepare but physiologically devastating to bone loss, cardiovascular health and obesity. A potential positive outcome of macronutrient selection was shown in the increased choice of protein in the H treated animals compared to S. This effect may be a positive influence in the anemic postmenopausal female with break-through bleeding. If the protein is consumed as red meat she will consume heme iron which will help to combat anemia. This research indicates that food choices may be affected by female sex hormones.

Empirically, body weight is different in the younger as opposed to the older human (Epstein, et. al., 1995). Lifestyles, intensity of exercise, and hormonal levels change with advancing years. The female sex hormones naturally produced in the human body cannot be duplicated in content or in metabolic physiological effects. Exogenous female sex hormones may provide

a supplement that will provide cardioprotection, protect against bone loss and improve the quality of life in females in this century and beyond. This is an opportunity not afforded to our grandmothers.

The changes in caloric conversion ratios reflect what could be happening in the human population. Body weight may be easier to maintain with endogenous hormones production as observed in the normally cycling S. Ovariectomy without supplemental hormones may produce added body weight as observed in the OV treated animals. After menopause or surgical removal of the ovaries, estrogen and E&P may help keep body weight at lower levels. Progesterone may create additional body weight that the body seeks (successfully temporally but unsuccessfully under the influence of poor nutrition) to lower through endogenous mechanisms not yet known.

The segment of the female population which motivated this research question is the female who reports additional cravings for chocolate during the luteal phase of the menstrual cycle when progesterone levels are elevated, the female taking oral contraceptives, and the postmenopausal female taking exogenous female sex hormones (Salhanic et., al.,1969). The progesterone treatment group tried to recover from the abrupt weight gain in Phase I. In phase II with essentially the same caloric intake the body changed the ratio at which calories were converted into body weight and the rate of gain was dramatically decreased. This looked hopeful, and yet under the influence of chocolate (a model for a poor nutritional choice), an unbalanced diet (termed poor nutrition), body weight crept back up and caloric conversion ratios converted very efficiently into increased body weight gain.

This pattern is also seen in the female in the human population. This is the physiological phenomonen which may have motivated the spending of the $33 billion dollars in weight loss reported by Dr. Richard Atkinson, current president of the Society for Clinical Nutrition (previously cited). Body weight increase is a major problem in our country. Despite the difficulties with treatment, Dr. Xavier Pi-Sunyer current president of the North American Society for the Study of Obesity, reports that 25% of the men and 45% of women are trying to lose weight at any one time. And that one in three adults in the US is currently attempting to lose weight (Pi-Sunyer, 1995). The answers to control are not currently known.

According to this study, the effects of exogenous female sex hormones on food intake and body weight gain in the aging female do not appear to contribute to the growing problem of obesity in this country. Typical regimes of hormone replacement (E or E&P) therapy do not produce increased body weight gain in the aging female rat. The perception that hormones cause excess body weight which would preclude the female from using HRT as a preventive measure against osteoporosis, cardiovascular disease and menopausal symptoms is not evident in these results. Hormonal effects on changes in caloric conversion ratios could indicate physiological and metabolic mechanisms which should be investigated in future research.

IMPLICATIONS FOR CLINICAL PRACTICE AND FUTURE RESEARCH

What do these results portend in terms of current body weight gain concepts reported across the television screens, popular press and taught in weight control clinics across the country? It may mean that there are metabolic regulators affecting body weight that go beyond merely counting calories. It

may mean that during certain times of the month in the normally cycling female, or the aging postmenopausal female taking cyclical HRT, food intake is converted into body weight at different ratios. Or it may mean that energy expenditure changes, or hormonally induced lethargy occurs or that a totally different concept exists that has not yet been fully discovered and reported.

As this research applies to the female human population, it is the desire of this researcher to propose new individualized assessments of dosage levels of HRT to protect against osteoporosis, cardiovascular disease and cancer. The risk assessments calculated with each female individually, based on her genetic predisposition to these diseases prior to prescribing exogenous supplements seems appropriate. She can then make decisions based on medical and scientific evidence. Negative symptoms of menopause which impact compliance rates of HRT are typically not measured in the human (Lerner, 1995). Further investigations are needed to measure hormonal effects on macronutrient food choice, caloric regulation, chocolate intake and subsequent impacts on body weight and body composition in the human.

Additionally, of interest are current National Health and Nutrition Examination Survey (NHANES III) reports that show 31.3% of men and 34.7% of women were found to be obese. The percentages for those Americans under the poverty line are even worse. In addition the prevalence for obesity has doubled since 1900 and increased 30% from 1980 to 1990 (Pi-Sunyer, 1995). There are those who would credit this national problem to decreased exercise and the "couch potato" syndrome. And yet, is there another unexplained hormonally influenced dimension which affects setpoint in body weight, causing calories to be converted into additional body weight at

different ratios? One of those differences recently reported in *Science* termed the hypothesis "a chronobiological solution" to metabolic alterations that affect obesity and non-insulin dependent diabetes. The process involves "resetting" hormonal rhythms to treat illness (Roush, 1995).

We have shown hormonal differences in the rate at which caloric intake is converted into body weight. Is the presence or absence of estrogen the only reason? This question would follow: if abnormal differences are detected, how can corrections be made, and can the source of manipulation be eliminated? The intake of dietary fat is clearly not the total picture as many current nutritionists report. The answer involves more than "willpower."

A recent answer to this question was revealed when researchers found that injections of the protein product of the mouse obesity gene caused both overweight and normal mice to lose weight. This new discovery called "leptin" is reported to provide dual action. It turns down the animals' appetites and increases their energy use, causing them to burn more fat. The parallel to the recent research is that mice with the same diets, treated with leptin lost 50% more weight than did the untreated animals, suggesting that food intake alone did not account for the weight loss. Two answers were given, the protein reduces the lethargy (increases activity) and speeds slow metabolisms. (Halaas, et. al., 1995). If ovariectomy slows the metabolism and produces a lethargy resulting in increased body weight, does this indicate unreported potential production of leptin by the ovary? Could there be a correlation between female sex hormones (estrogen and progesterone) and the rate at which the leptin is produced in the body. Does the prolactin hormone and resetting of chronological clocks play a role in the mechanisms of leptin and

the way estrogen and progesterone affect body weight? The altering of circadian rhythms (which may impact all metabolic regulators) may be a solution to uncontrolled body weight gain, diabetes and other disease states (Roush, 1995). Clearly there are mechanisms which are currently active in the aging female rat that are not understood or reported.

Caloric regulation and eating patterns clearly need to be evaluated. Fat and protein intake may provide satiating factors that offset the uncontrolled consumption of highly refined, high carbohydrate snack foods. Total caloric intake as compared to the percentage of caloric intake derived from fat may be misinterpreted by many consumers. Increased dietary fat and protein levels in this study did not increase body weight proportionately. Rather, higher levels of carbohydrate intake were positively correlated to body weight gain and higher fat and protein intake to less body weight gain.

Why does the body choose to convert calories into body weight at different ratios? Is this evidence of the setpoint hypothesis? Is this system which has potentially worked well for centuries being altered by exogenous progestins, estrogens, menopause or endogenous progesterone, which could cause setpoint to be altered in unexpected, unpredicted and undesirable ways? Is all of this the result of a biological "survival of the species" which provides additional energy during the luteal phase when the body is potentially preparing to support new life? This study contributes new information in an aging model into the investigation of female body weight gain, which will hopefully provide a basis for more extensive investigation in the future and provide further information to this perplexing question. In clinics

and future research studies that involve the aging female the following implications should be considered:

1. The hormonal phase (endogenous levels) in the normally cycling female may affect food intake and body weight data, and should therefore be measured and documented in all future research studies.
2. The postmenopausal woman could emerge as the model for examining female health parameters. The female postbreeder rat can provide preliminary data in a controlled environment.
3. There may be metabolic differences in the way calories are converted into body weight, which may be affected by both endogenous and exogenous female sex hormones.
4. Eating behavior, food choices and caloric regulation may be affected by endogenous and exogenous female sex hormones. Higher protein intake in the postmenopausal female with breakthrough bleeding can be of value if she is deficient in heme iron and consumes red meat.
5. Postmenopausal women on hormone replacement therapy may experience different caloric conversion ratios depending upon the nature, combination and dosage of the exogenous female sex hormones. Progesterone caused early higher rate of body weight gain which decreased with time. Therefore at the onset of menopause or immediately following surgery when body weight gain most often occurs, progesterone should be prescribed in minimal dosages known to be protective against cancer.

6. Various levels and sources of exogenous hormones in addition to various levels of endogenous female sex hormones may affect food intake and body weight and should therefore be monitored. Dosages should be individualized based on risk assessment ratios.
7. Body weight changes in menopause may occur as the estrogen pool declines. In the future, perimenopausal females could suppress midlife body weight gain by beginning HRT earlier.
8. New research is needed to "reset" the setpoint when stress, genetic or environmental factors violate or disturb this hypothesized metabolic mechanism (Roush, 1995).
9. Further research is needed to examine the effects of hormone replacement therapy on cardiovascular disease, cancer, diabetes mellitus and other related diseases in the postmenopausal woman.
10. In the aging female model the absence of HRT with access to novel food choices and chocolate (with or without HRT) contributed most to body weight gain. The data from the present study indicate that hormone replacement therapy in levels typically prescribed to deter menopausal conditions in midlife do not necessarily contribute to additional body weight gain.

The larger question encompassing all previously discussed parameters is: what determines body weight fluctuations? Body weight gain in menopause as it relates to hormonal profiles has been implied. This research does not agree with earlier findings in the younger rat model which show additional food intake resulting in additional body weight gain and a progesterone effect with an accompanying increase in percent body fat stores.

A recent commentary on classics in obesity by Dr. George Bray (1995) examines the possibility that human beings might defy the first law of thermodynamics (termed "Luxuskonsumption") by being more of less efficient in the conversion of calories into body weight. In the early 1900's male scientists studied the concept carefully using themselves as subjects with careful feeding and recording of body weight with differing results. Additional and more current studies show a linear relationship in the degree of weight gain of overfeeding both male and female subjects (Forbes, et al., 1986). However, the measurement of endogenous female hormone levels, age and time is not evident in the reported results. These parameters may affect energy expenditure reported by Leibel et. al. (1995) and sympathetic activity modulating to variable degrees the nutrient partitioning between carbohydrate storage and metabolism and protein storage and catabolism with fat storage being the pathway consuming the residual surplus energy (Bray, 1995). This current research in the hormonal effects in the postbreeder female rat, reveals that these parameters should be measured and documented in future research. Another analysis of "Luxuskonsumption" in the human female should be conducted to report documented hormonal effects.

The highly refined carbohydrate diet that is advertised and marketed so convincingly across the globe is the same product that Dr. Paula Geiselman stated in 1987 would stimulate appetite. If the postbreeder female rat is a model that parallels what could be happening in the postmenopausal female, this research documents her findings. Highly refined carbohydrate foods may stimulate body weight gain and possibly appetite. The wide spread pattern of excess food consumption in a population motivated by convenience may

contribute to the increase in insulin resistance and body weight gain. Satiety may not be achieved at the same level as with fat. The population consuming highly refined carbohydrate diets deficient in nutritious satiating foods with resulting obesity, should be further investigated. The increase in Type II diabetes and insulin resistance in this country with regard to the consumption of highly refined carbohydrate diets is a growing concern. The question is: is a calorie a calorie in all physiological anatomical states. Is a calorie a calorie with all foods or do some macronutrients convert at different rates? Age, surgical implications, metabolic mechanisms and hormonal effects on eating behavior may reach beyond the female taking exogenous female sex hormones.

Data from the recent PEPI trial and the Women's Health Initiative to be completed in the year 2005 (Healy, 1993) may provide new evidence to correlate current research findings in the human female. Perhaps this research can provide a small glimpse into what has been termed a major health problem, the uncontrolled increase of body weight in this country in a growing segment of the population. The larger segment of the population who maintain healthy life style patterns should be commended.

The effects of exogenous female sex hormones on food intake and body weight gain in the aging female do not appear to contribute to the growing problem of obesity in this country. Genetic and environmental influences on hormonal effects of changes in caloric conversion ratios (also termed luxuskonsumption) indicate physiological and metabolic mechanisms which should be investigated in future research.

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APPENDIX A PHASE I

Phase I ANOVA(4-groups) Body weight

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Treatment	3	2952.500	984.167	9.240	.0001
Residual	39	4154.012	106.513		

Dependent: Body Weight

Phase I ANOVA(4-groups) Body Weight %

Dependent: Wt % Ph1

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Treatment	3	373.914	124.638	8.779	.0001
Residual	39	553.702	14.197		

Phase I ANOVA(4-groups) Body WEIGHT %

Ovariec vs Hormones
Effect: Treatment
Dependent: Wt % Ph1

	Cell Weight
Ovariec	1.000
Ovar-E&P	-.333
Ovar-E	-.333
Ovar-P	-.333

df	1
Sum of Squares	77.228
Mean Square	77.228
F-Value	5.440
P-Value	.0249

Estrogen vs No Estrogen
Effect: Treatment
Dependent: Wt % Ph1

	Cell Weight
Ovariec	-.500
Ovar-E&P	.500
Ovar-E	.500
Ovar-P	-.500

df	1
Sum of Squares	328.988
Mean Square	328.988
F-Value	23.172
P-Value	.0001

Phase I ANOVA(2x2 Factorial) Body weight

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Estrogen	1	2562.836	2562.836	24.061	.0001
Progesterone	1	353.567	353.567	3.319	.0761
Estrogen * Progesterone	1	69.583	69.583	.653	.4238
Residual	39	4154.012	106.513		

Dependent: Body Weight

ANOVA for Initial Body Wt

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Treatment	4	2223.235	555.809	1.056	.3881
Residual	50	26318.111	526.362		

Phase I ANOVA(2x2 Factorial) Total Calories

Dependent: Total Calories

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Estrogen	1	83624.884	83624.884	3.949	.0540
Progesterone	1	4910.826	4910.826	.232	.6328
Estrogen * Progesterone	1	14382.587	14382.587	.679	.4149
Residual	39	825891.562	21176.707		

Phase I ANOVA(4-groups) Caloric Conversion

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Treatment	3	3059.814	1019.938	8.740	.0001
Residual	39	4551.407	116.703		

Dependent: Caloric Conversion

Phase I ANOVA (4-groups) Caloric Conversion

Ovariec vs Hormones
Effect: Treatment
Dependent: Feed Efficiency

	Cell Weight
C-Ovariec	1.000
E-Ovar-E&P	-.333
D-Ovar-E	-.333
F-Ovar-P	-.333

df	1
Sum of Squares	665.768
Mean Square	665.768
F-Value	5.705
P-Value	.0219

Estrogen vs No Estrogen
Effect: Treatment
Dependent: Feed Efficiency

	Cell Weight
C-Ovariec	-.500
E-Ovar-E&P	.500
D-Ovar-E	.500
F-Ovar-P	-.500

df	1
Sum of Squares	2713.328
Mean Square	2713.328
F-Value	23.250
P-Value	.0001

Phase I ANOVA(2x2 Factorial) Caloric Conversion

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Estrogen	1	2713.328	2713.328	23.250	.0001
Progesterone	1	275.162	275.162	2.358	.1327
Estrogen * Progesterone	1	104.109	104.109	.892	.3507
Residual	39	4551.407	116.703		

Dependent: Feed efficiency

Phase I ANOVA(2x2 Factorial) Fat

Dependent: Fat**Type III**
Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Estrogen	1	1370.862	1370.862	.065	.7999
Progesterone	1	110195.286	110195.286	5.237	.0276
Estrogen * Progesterone	1	9949.363	9949.363	.473	.4958
Residual	39	820674.081	21042.925		

Phase I ANOVA(2x2 Factorial) CHO

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Estrogen	1	192654.816	192654.816	10.274	.0027
Progesterone	1	92706.323	92706.323	4.944	.0320
Estrogen * Progesterone	1	13725.693	13725.693	.732	.3975
Residual	39	731325.542	18751.937		

Dependent: Carbohydrate

APPENDIX B PHASE II

Phase II ANOVA(5-groups) Body Weight

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
TREATMENT	4	937.331	234.333	3.795	.0090
Residual	50	3087.778	61.756		

Dependent: BODY WT

Phase II ANOVA(5-groups) Body Weight %

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Treatment	4	111.394	27.848	3.576	.0122
Residual	50	389.412	7.788		

Dependent: Wt % Ph2

Phase II ANOVA (5-groups) Body Weight %

Ovar vs Hormones
Effect: Treatment
Dependent: Wt % Ph2

	Cell Weight
Ovariec	1.000
Ovar-E&P	-.333
Ovar-E	-.333
Ovar-P	-.333

df	1
Sum of Squares	82.507
Mean Square	82.507
F-Value	10.594
P-Value	.0020

Prog vs No Prog
Effect: Treatment
Dependent: Wt % Ph2

	Cell Weight
Ovariec	-.500
Ovar-E&P	.500
Ovar-E	-.500
Ovar-P	.500

df	1
Sum of Squares	67.785
Mean Square	67.785
F-Value	8.703
P-Value	.0048

Phase II ANOVA(2x2 Factorial) Body weight

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Estrogen	1	152.253	152.253	2.481	.1233
Progesterone	1	507.751	507.751	8.274	.0065
Estrogen * Progesterone	1	184.751	184.751	3.011	.0906
Residual	39	2393.278	61.366		

Dependent: Body-WT

Phase II ANOVA(5-groups) Total Calories

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
TREATMENT	4	476458.703	119114.676	6.501	.0003
Residual	50	916171.149	18323.423		

Dependent: TOTAL-CAL

Phase II ANOVA(2x2 Factorial) Caloric Conversion

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Estrogen	1	108.027	108.027	1.598	.2137
Progesterone	1	501.476	501.476	7.416	.0096
Estrogen * Progesterone	1	211.395	211.395	3.126	.0849
Residual	39	2637.060	67.617		

Dependent: Caloric Conversion

Phase II ANOVA(2x2 Factorial) Protein

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Estrogen	1	1344.946	1344.946	1.584	.2156
Progesterone	1	216.557	216.557	.255	.6163
Estrogen * Progesterone	1	16958.511	16958.511	19.979	.0001
Residual	39	33104.202	848.826		

Dependent: Protein

Phase II ANOVA(2x2 Factorial) CHO(SW&NSW)

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Estrogen	1	218089.292	218089.292	10.259	.0027
Progesterone	1	53816.213	53816.213	2.532	.1197
Estrogen * Progesterone	1	35135.194	35135.194	1.653	.2062
Residual	39	829046.029	21257.590		

Dependent: Total-CHO

Phase II ANOVA(2x2 Factorial) CHO/NSW

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Estrogen	1	149382.849	149382.849	9.582	.0036
Progesterone	1	20846.903	20846.903	1.337	.2546
Estrogen * Progesterone	1	8779.832	8779.832	.563	.4575
Residual	39	608000.548	15589.758		

Dependent: CHO/NSW

Phase II ANOVA(2x2 Factorial) CHO/SW

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Estrogen	1	6480.634	6480.634	.832	.3674
Progesterone	1	7673.502	7673.502	.985	.3271
Estrogen * Progesterone	1	79042.356	79042.356	10.144	.0028
Residual	39	303888.087	7792.002		

Dependent: CHO/SW

APPENDIX C PHASE III

Phase III ANOVA(5-groups) Body Weight

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Treatment	4	4744.030	1186.008	12.048	.0001
Residual	50	4921.970	98.439		

Dependent: Ph3 Body Wt

Phase III ANOVA(5-groups) Body Weight %

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Treatment	4	567.626	141.907	12.905	.0001
Residual	50	549.802	10.996		

Dependent: Wt % Ph3

Phase III ANOVA (5 groups) body weight %

Sham vs Hormone
Effect: Treatment
Dependent: Wt % Ph3

	Cell Weight
SHAM	1.000
Ovar-E&P	-.333
Ovar-E	-.333
Ovar-P	-.333

df	1
Sum of Squares	558.094
Mean Square	558.094
F-Value	50.754
P-Value	.0001

Phase III ANOVA(5-groups) Total Calories

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Treatment	4	733829.624	183457.406	9.161	.0001
Residual	50	1001249.381	20024.988		

Dependent: Ph3 Total Cal

Phase III ANOVA(2x2 Factorial) Protein

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Estrogen	1	444.814	444.814	.291	.5924
Progesterone	1	785.464	785.464	.515	.4774
Estrogen * Progesterone	1	10557.041	10557.041	6.916	.0122
Residual	39	59533.528	1526.501		

Dependent: PROTEIN

Phase III ANOVA(2x2 Factorial) HF/LS

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Estrogen	1	102.879	102.879	.104	.7494
Progesterone	1	6306.571	6306.571	6.345	.0160
Estrogen * Progesterone	1	2416.334	2416.334	2.431	.1270
Residual	39	38765.961	993.999		

Dependent: HF/LS

APPENDIX D TOTAL STUDY

All Phases ANOVA(4-groups) Body Weight

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Treatment	3	3358.855	1119.618	3.849	.0166
Residual	39	11343.087	290.848		

Dependent: Total Weight

All Phases ANOVA(4-groups) Body Weight

Ovariec vs Hormones
Effect: Treatment
Dependent: Total Weight

	Cell Weight
Ovariec	1.000
Ovar-E&P	-.333
Ovar-E	-.333
Ovar-P	-.333

df	1
Sum of Squares	1495.316
Mean Square	1495.316
F-Value	5.141
P-Value	.0290

Estrogen vs No Estrogen
Effect: Treatment
Dependent: Total Weight

	Cell Weight
Ovariec	-.500
Ovar-E&P	.500
Ovar-E	.500
Ovar-P	-.500

df	1
Sum of Squares	3199.449
Mean Square	3199.449
F-Value	11.000
P-Value	.0020

ALL PHASES ANOVA (4 GROUPS) BODY WEIGHT%

Ovariec vs Hormones
Effect: Treatment
Dependent: TotlWt %

	Cell Weight
Ovariec	1.000
Ovar-E&P	-.333
Ovar-E	-.333
Ovar-P	-.333

df	1
Sum of Squares	221.496
Mean Square	221.496
F-Value	5.311
P-Value	.0266

Estrogen vs No Estrogen
Effect: Treatment
Dependent: TotlWt %

	Cell Weight
Ovariec	-.500
Ovar-E&P	.500
Ovar-E	.500
Ovar-P	-.500

df	1
Sum of Squares	352.782
Mean Square	352.782
F-Value	8.459
P-Value	.0060

All Phases ANOVA(2x2 Factorial) Total Body weight

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Estrogen	1	3579.215	3579.215	12.656	.0010
Progesterone	1	18.766	18.766	.066	.7981
Estrogen * Progesterone	1	99.631	99.631	.352	.5562
Residual	39	11029.767	282.815		

Dependent: Total Weight

All Phases ANOVA(4-groups) Caloric Conversion

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Treatment	3	289.708	96.569	2.704	.0585
Residual	39	1392.639	35.709		

Dependent: Feed Efficiency

ALL Phases ANOVA (4-groups) Caloric Conversion

Estrogen vs No Estrogen
Effect: Treatment
Dependent: Feed Efficiency

	Cell Weight
Ovarlec	-.500
Ovar-E&P	.500
Ovar-E	.500
Ovar-P	-.500

df	1
Sum of Squares	281.835
Mean Square	281.835
F-Value	7.893
P-Value	.0077

All Phases ANOVA(2x2 Factorial) Caloric Conversion

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Estrogen	1	326.781	326.781	9.421	.0039
Progesterone	1	.072	.072	.002	.9639
Estrogen * Progesterone	1	2.645	2.645	.076	.7839
Residual	39	1352.763	34.686		

Dependent: Feed Efficiency

All Phases ANOVA(4-groups) Total Protein

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Treatment	3	137560.651	45853.550	4.764	.0063
Residual	39	375344.564	9624.220		

Dependent: Total Pro

Scheffe's S
Effect: Treatment
Dependent: Total Pro
Significance level: .05

	Vs.	Diff.	Crit. diff.	P-Value	
Ovar-P	Ovar-E	22.145	125.228	.9657	
	Ovariec	85.519	128.175	.2992	
	Ovar-E&P	141.865	122.719	.0175	S
Ovar-E	Ovariec	63.374	125.228	.5411	
	Ovar-E&P	119.720	119.637	.0498	S
Ovariec	Ovar-E&P	56.346	122.719	.6190	

S = Significantly different at this level.

All Phases ANOVA(2x2 Factorial) Total Protein

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Estrogen	1	7417.964	7417.964	.700	.4078
Progesterone	1	1775.098	1775.098	.168	.6845
Estrogen * Progesterone	1	85279.127	85279.127	8.052	.0072
Residual	39	413074.880	10591.664		

Dependent: Total Pro

All Phases ANOVA(4-groups) Total CHO

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Treatment	3	1259774.125	419924.708	3.438	.0260
Residual	39	4763878.096	122150.720		

Dependent: Total CHO

ALL PHASES ANOVA (4-groups) Total CHO

Ovariec vs Hormones
Effect: Treatment
Dependent: Total CHO

	Cell Weight
Ovariec	1.000
Ovar-E&P	-.333
Ovar-E	-.333
Ovar-P	-.333

df	1
Sum of Squares	1036668.197
Mean Square	1036668.197
F-Value	8.487
P-Value	.0059

Estrogen vs No Estrogen
Effect: Treatment
Dependent: Total CHO

	Cell Weight
Ovariec	-.500
Ovar-E&P	.500
Ovar-E	.500
Ovar-P	-.500

df	1
Sum of Squares	921529.914
Mean Square	921529.914
F-Value	7.544
P-Value	.0091

All Phases ANOVA(2x2 Factorial) Total CHO

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Estrogen	1	1259595.510	1259595.510	10.870	.0021
Progesterone	1	154576.336	154576.336	1.334	.2551
Estrogen * Progesterone	1	48634.523	48634.523	.420	.5209
Residual	39	4519195.579	115876.810		

Dependent: Total CHO

APPENDIX E POST MORTEM

ANOVA(4-groups): Progesterone

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
TREAT	3	1437.465	479.155	3.304	.0304
Residual	38	5510.928	145.024		

Dependent: PROG

ANOVA (4-GROUPS): TESTOSTERONE

Progest vs No Progest
Effect: TREAT
Dependent: TEST

	Cell Weight
C-Ovariec	-.500
E-Ovar-E&P	.500
D-Ovar-E	-.500
F-Ovar-P	.500

df	1
Sum of Squares	2.094
Mean Square	2.094
F-Value	4.063
P-Value	.0508

ANOVA(4-groups): Wt of Uterus

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Treatment	3	1.053	.351	17.710	.0001
Residual	39	.773	.020		

Dependent: Wt of Uterus

Scheffe's S
Effect: Treatment
Dependent: Wt of Uterus
Significance level: .05

	Vs.	Diff.	Crit. diff.	P-Value	
C-Ovariec	F-Ovar-P	.160	.184	.1092	
	E-Ovar-E&P	.372	.176	.0001	S
	D-Ovar-E	.376	.180	.0001	S
F-Ovar-P	E-Ovar-E&P	.212	.176	.0123	S
	D-Ovar-E	.216	.180	.0125	S
E-Ovar-E&P	D-Ovar-E	.004	.172	.9999	

S = Significantly different at this level.

ANOVA (4-GROUPS) WT OF UTERUS

Ovariec vs Hormones
Effect: Treatment
Dependent: Wt of Uterus

	Cell Weight
C-Ovariec	1.000
E-Ovar-E&P	-.333
D-Ovar-E	-.333
F-Ovar-P	-.333

df	1
Sum of Squares	.703
Mean Square	.703
F-Value	35.443
P-Value	.0001

Estrogen vs No Estrogen
Effect: Treatment
Dependent: Wt of Uterus

	Cell Weight
C-Ovariec	-.500
E-Ovar-E&P	.500
D-Ovar-E	.500
F-Ovar-P	-.500

df	1
Sum of Squares	.925
Mean Square	.925
F-Value	46.656
P-Value	.0001

ANOVA(2x2 Factorial): Wt of Uterus

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Estrogen	1	.925	.925	46.656	.0001
Progesterone	1	.072	.072	3.626	.0643
Estrogen * Progesterone	1	.065	.065	3.279	.0779
Residual	39	.773	.020		

Dependent: Wt of Uterus

ANOVA(2x2 Factorial): Fat % of Liver

Type III Sums of Squares

Source	df	Sum of Squa...	Mean Squa...	F-Value	P-Value
Estrogen	1	4.955	4.955	6.310	.0163
Progesterone	1	.083	.083	.105	.7471
Estrogen * Progestero...	1	.267	.267	.340	.5634
Residual	39	30.625	.785		

Dependent: %FAT OF LIVER

ANOVA(2x2 Factorial): Weight/Length ratio

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Estrogen	1	17.434	17.434	8.577	.0057
Progesterone	1	.651	.651	.320	.5748
Estrogen * Progesterone	1	.487	.487	.240	.6272
Residual	39	79.277	2.033		

Dependent: W/L ratio

APPENDIX F SUMMARY FIGURES

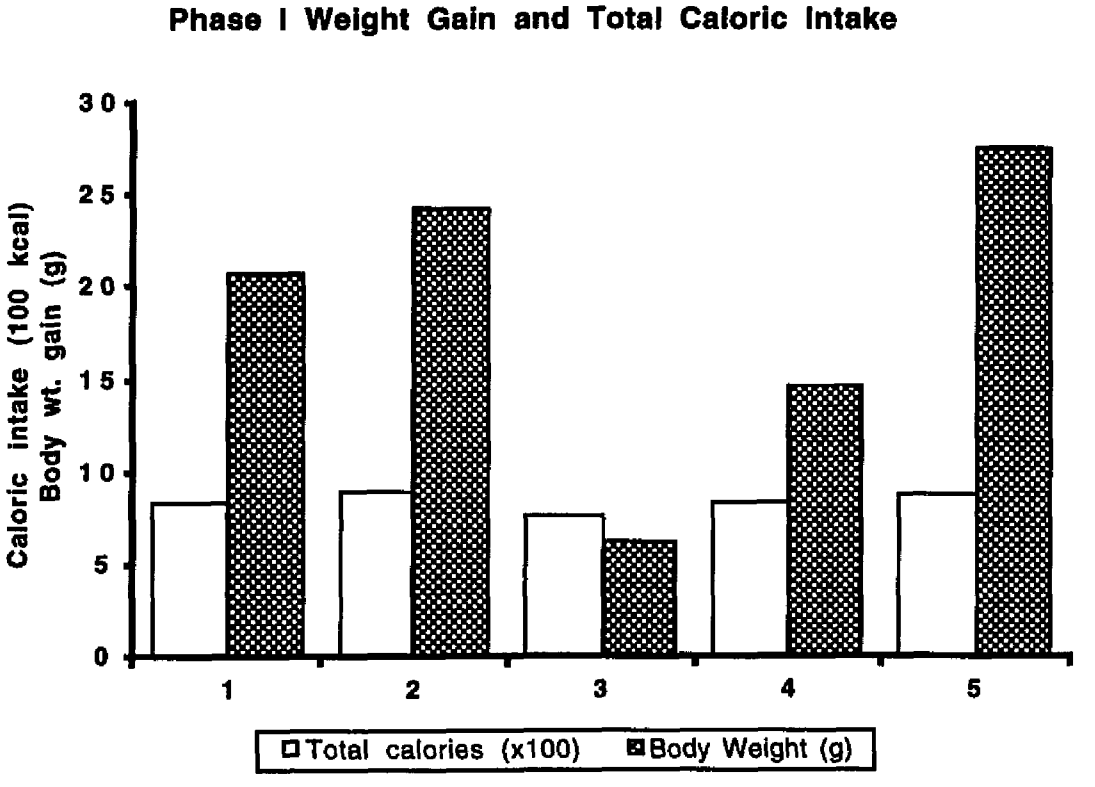

Figure 1.0 Phase I Weight Gain and Total Caloric Intake
Treatment: 1. Sham, 2. No hormone, 3. Estrogen, 4. Estrogen and Progesterone, 5. Progesterone. 2-5 were ovariectomized.

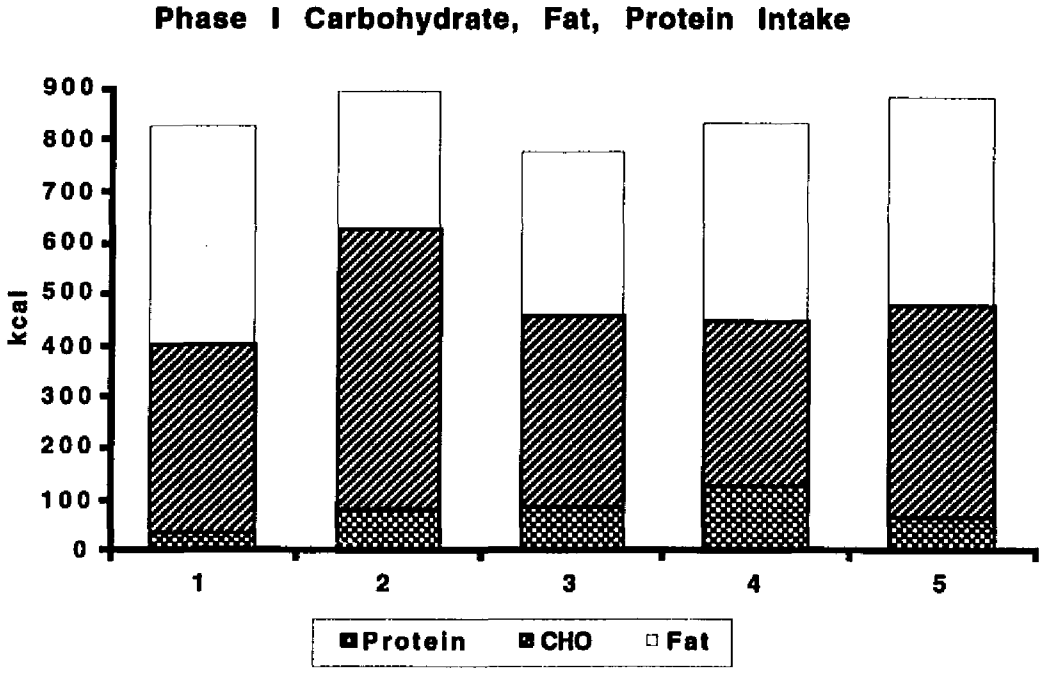

Figure 2.0 Phase I Carbohydrate, Fat, Protein Intake
Treatment: 1. Sham, 2. No hormone, 3. Estrogen, 4. Estrogen and Progesterone, 5. Progesterone. 2-5 were ovariectomized.

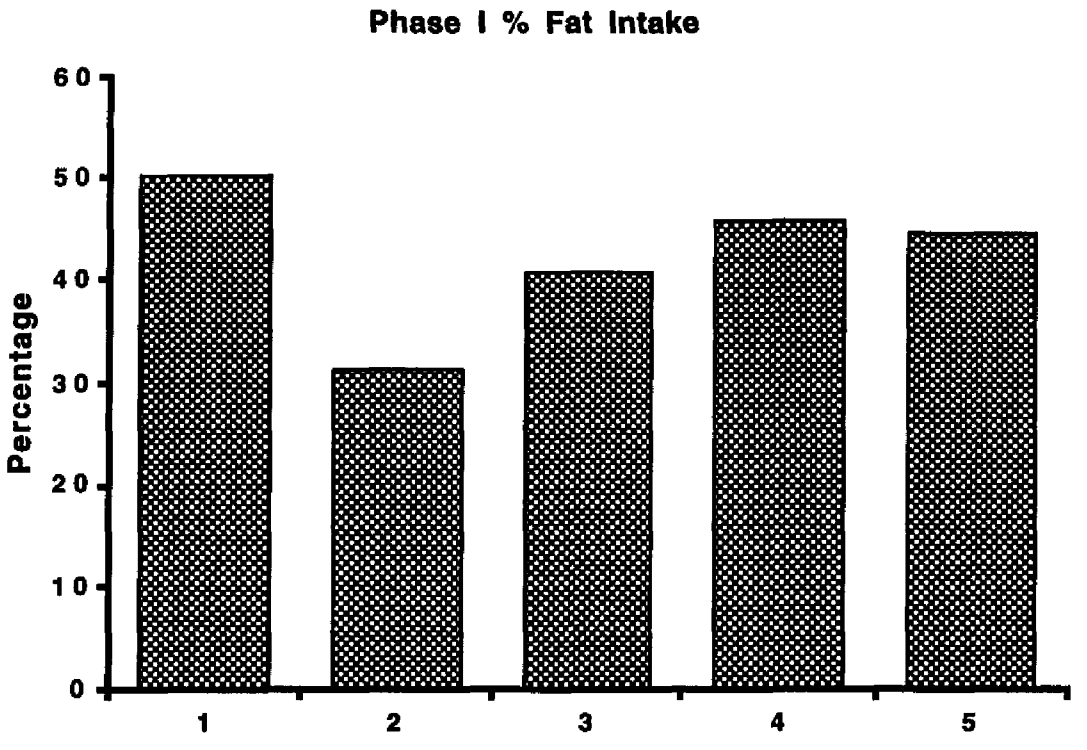

Figure 3.0 Phase I % Fat Intake
Treatment: 1. Sham, 2. No hormone, 3. Estrogen, 4. Estrogen and Progesterone, 5. Progesterone. 2-5 were ovariectomized.

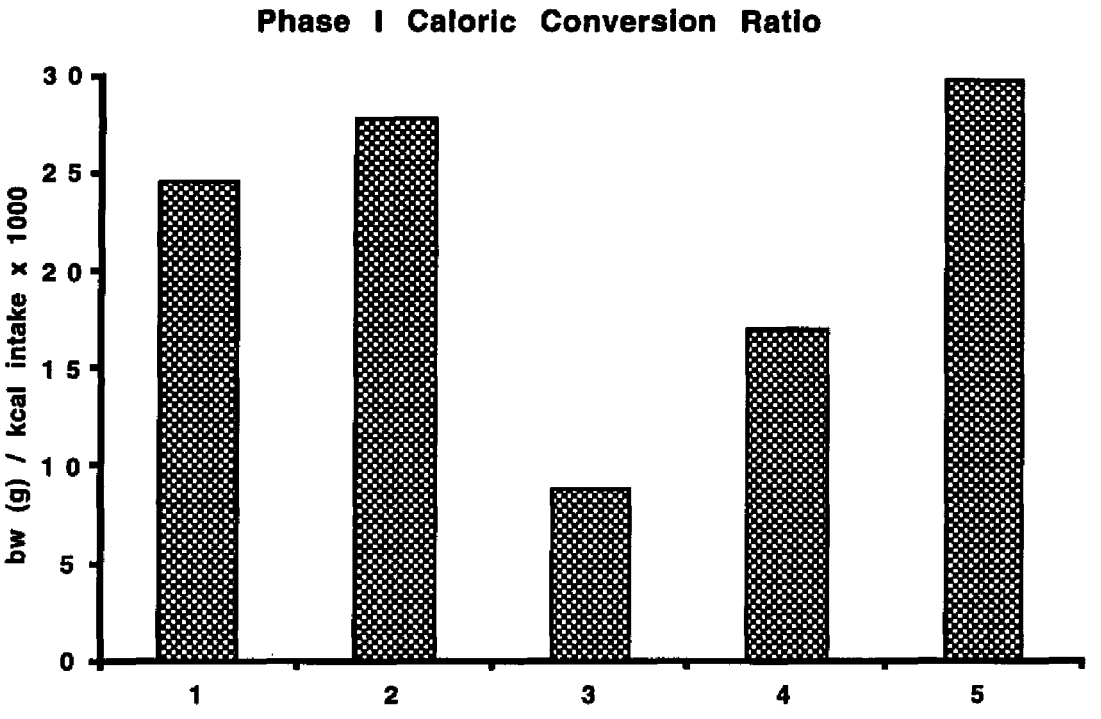

Figure 4.0 Phase I Caloric Conversion Ratio
Treatment: 1. Sham, 2. No hormone, 3. Estrogen, 4. Estrogen and Progesterone, 5. Progesterone. 2-5 were ovariectomized

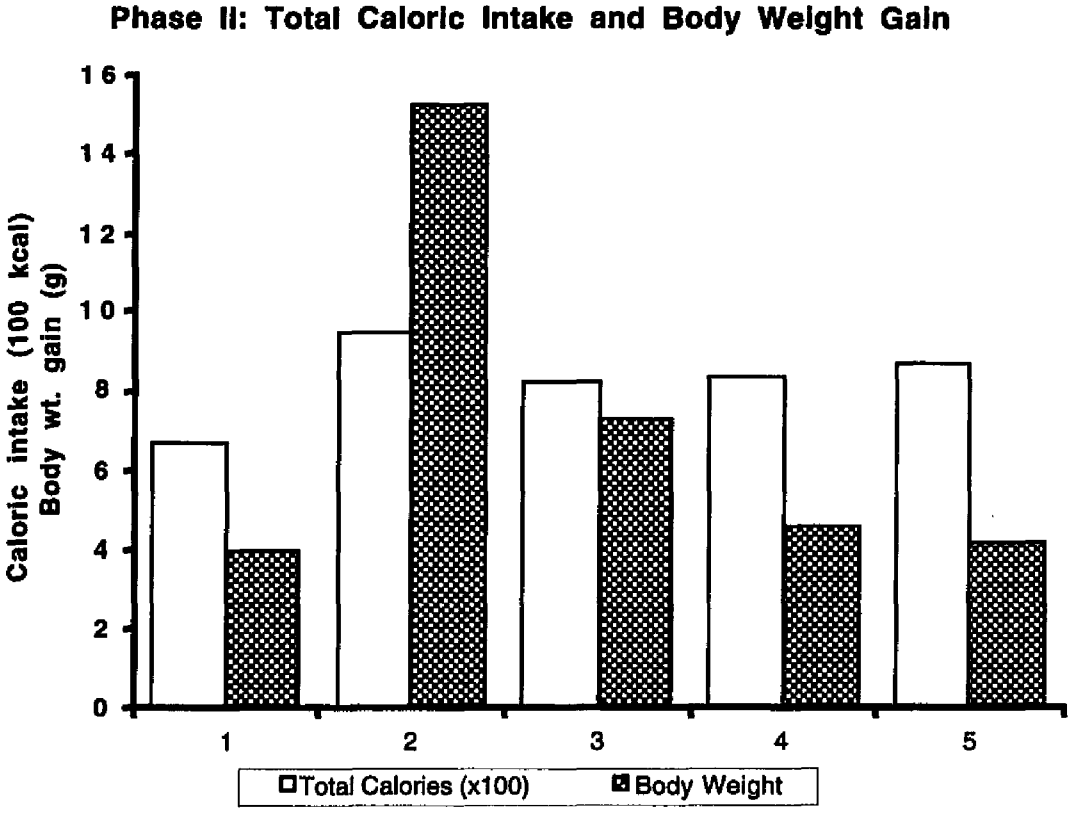

Figure 5.0 Phase II: Total Caloric Intake and Body Weight Gain
Treatment: 1. Sham, 2. No hormone, 3. Estrogen, 4. Estrogen and Progesterone, 5. Progesterone. 2-5 were ovariectomized.

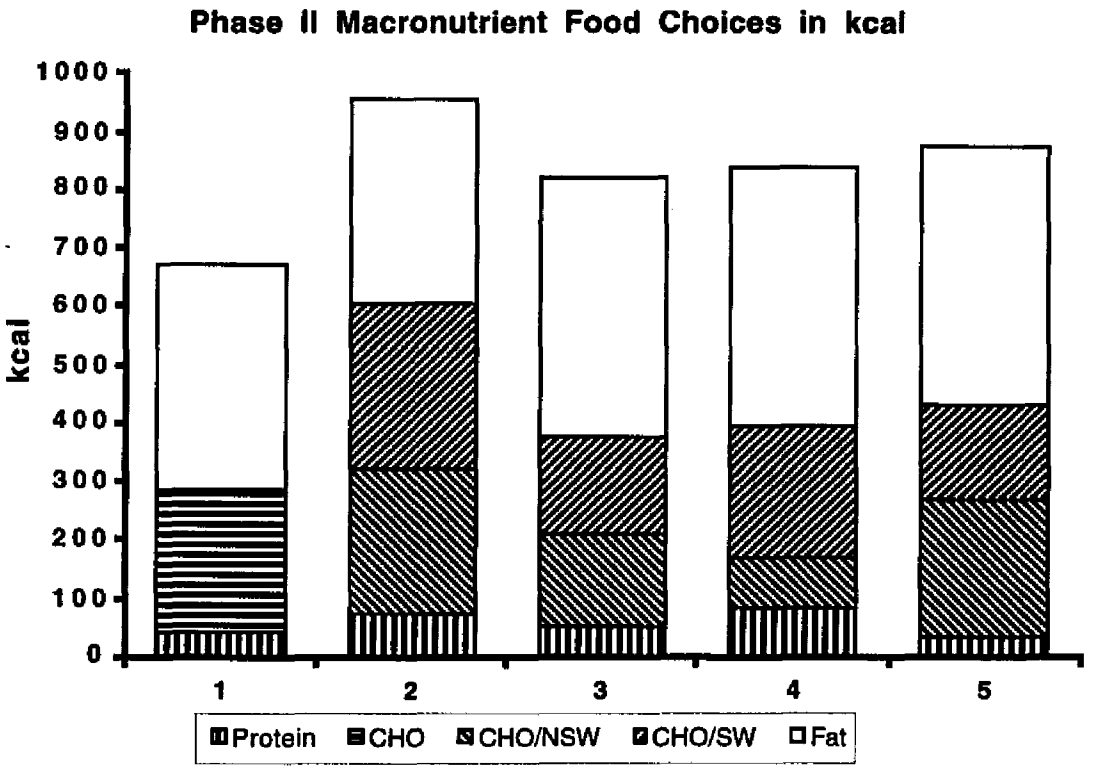

Figure 6.0 Phase II Macronutrient Food Choices in kcal
Treatment: 1. Sham, 2. No hormone, 3. Estrogen, 4. Estrogen and Progesterone, 5. Progesterone. 2-5 were ovariectomized.

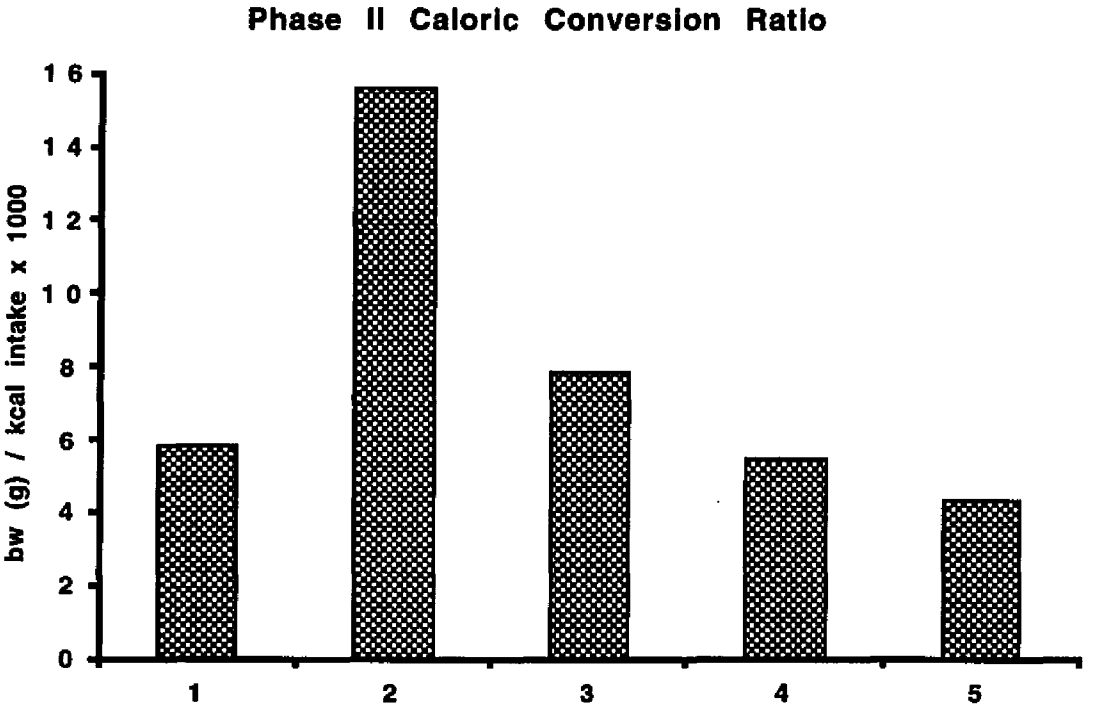

Figure 7.0 Phase II Caloric Conversion Ratio
Treatment: 1. Sham, 2. No hormone, 3. Estrogen, 4. Estrogen and Progesterone, 5. Progesterone. 2-5 were ovariectomized.

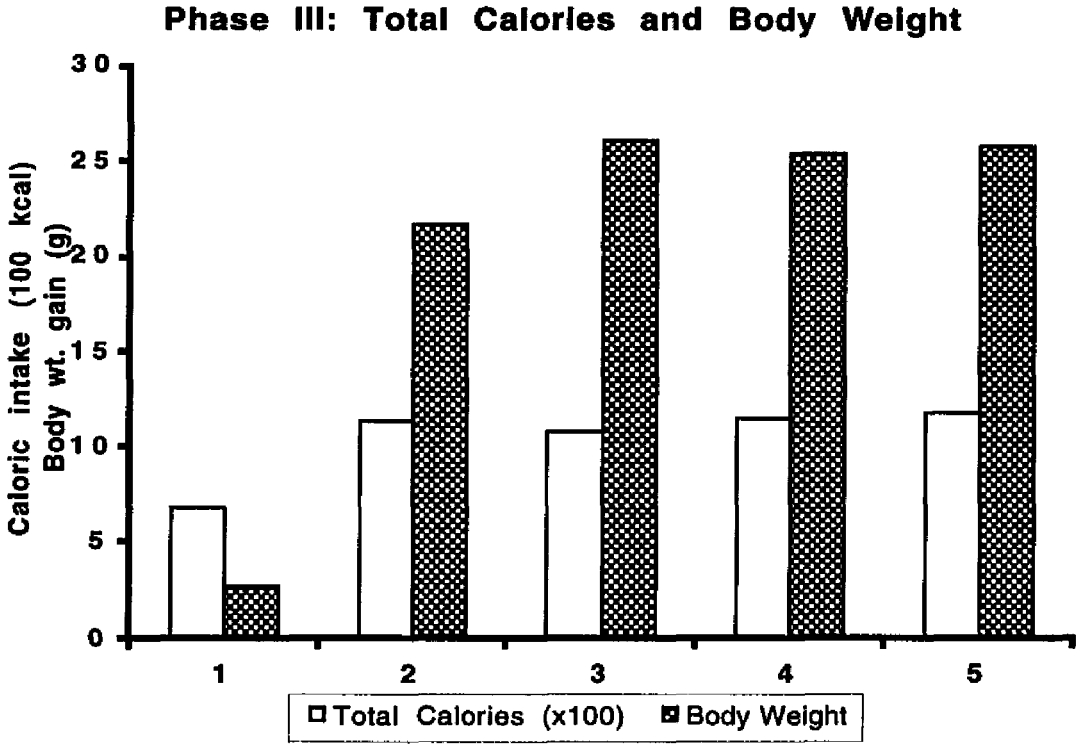

Figure 8.0 Phase III: Total Calories and Body Weight
Treatment: 1. Sham, 2. No hormone, 3. Estrogen, 4. Estrogen and Progesterone, 5. Progesterone. 2-5 were ovariectomized

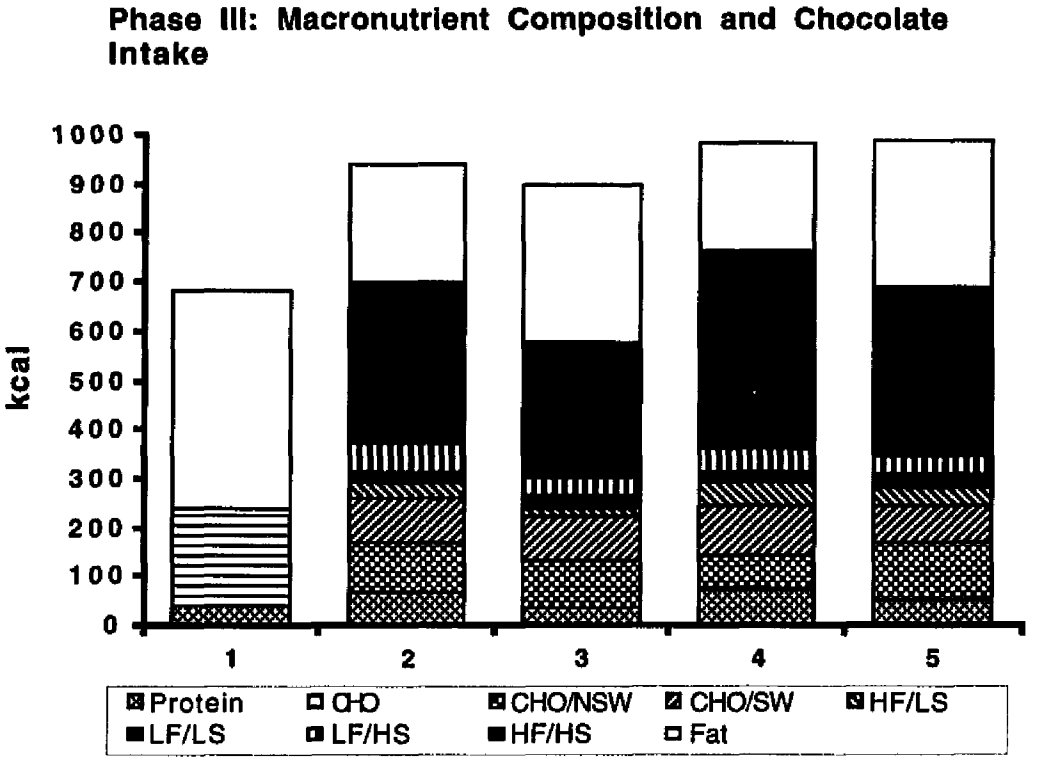

Figure 9.0 Phase III: Macronutrient Composition and Chocolate Intake Treatment: 1. Sham, 2. No hormone, 3. Estrogen, 4. Estrogen and Progesterone, 5. Progesterone. 2-5 were ovariectomized. Sham was not given chocolate sources.

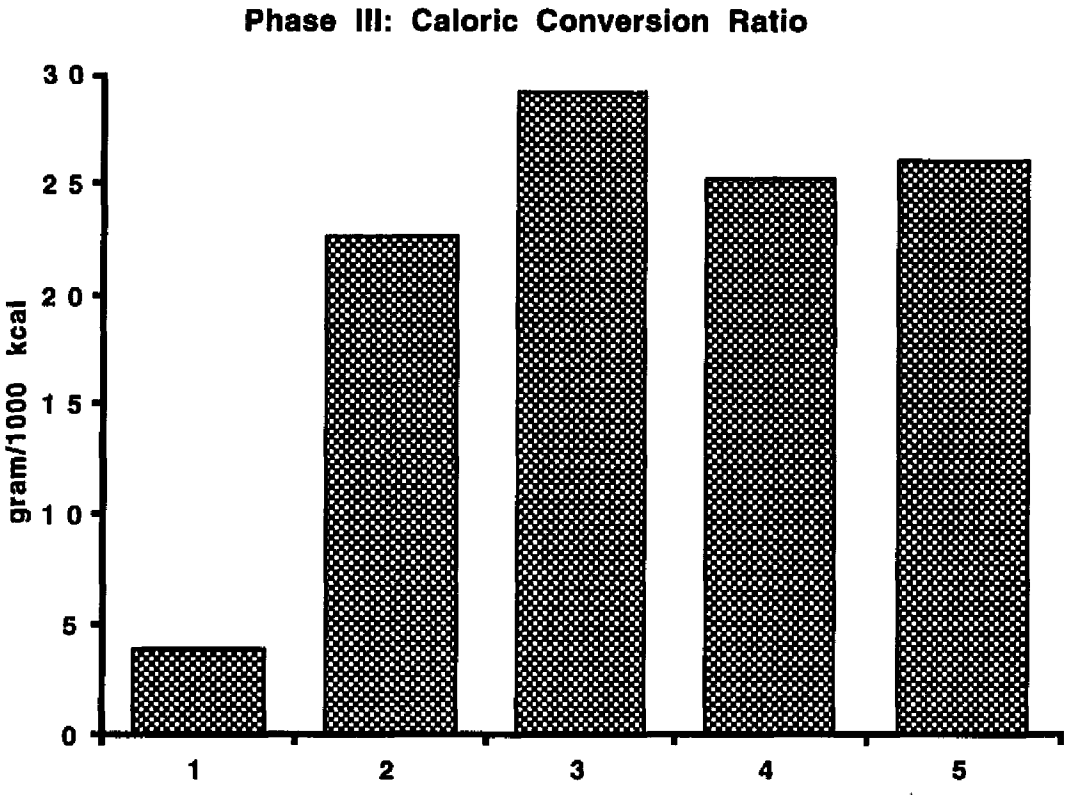

Figure 10.0 Phase III: Caloric Conversion Ratio
Treatment: 1. Sham, 2. No hormone, 3. Estrogen, 4. Estrogen and Progesterone, 5. Progesterone. 2-5 were ovariectomized.

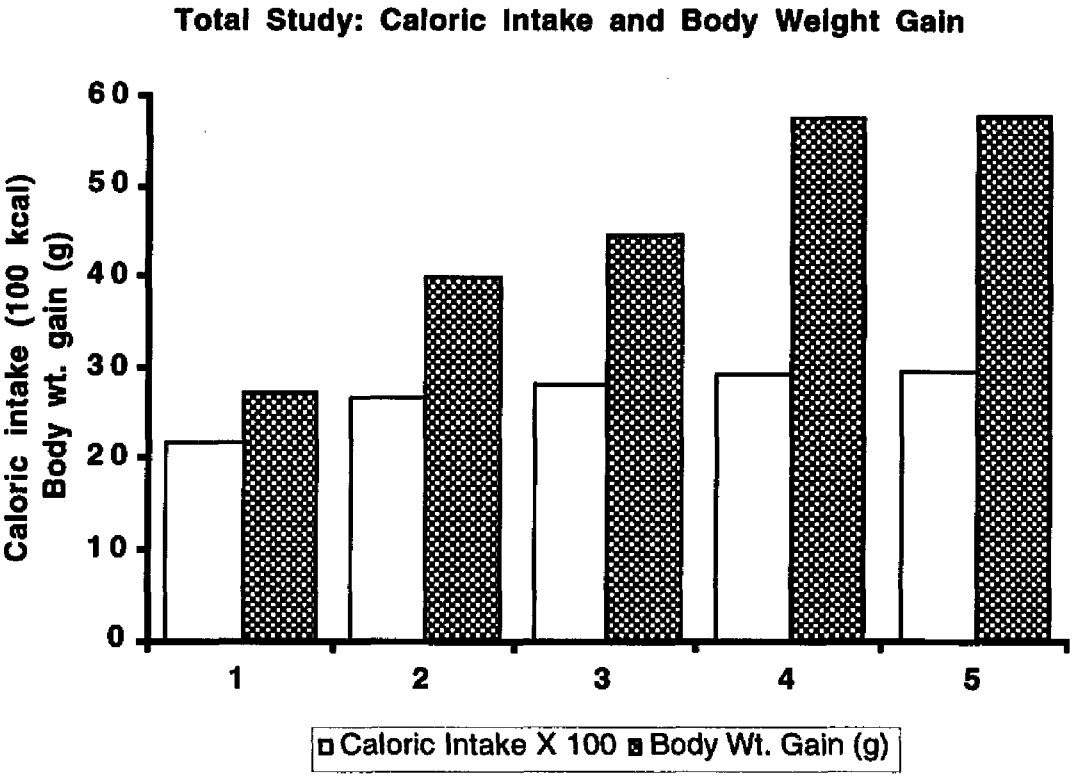

Figure 11.0 Total study: Caloric Intake and Body Weight Gain
Treatment 1. Sham, 2. Estrogen, 3. Estrogen with Progesterone, 4. Progesterone only, 5. Ovariectomized with no hormone. 2-5 were ovariectomized. Grams of body weight increase compared to caloric intake.

Group	Grams consumed	Body Weight Gained (g):
Sham	2184±69	27.3±4.3
Estrogen	2493 ±83	39.9±5.19
Estrogen & Prog.	2650±93	44.63.19
Progesterone	2743±100	57.7±5.96
Ovariectomized	2794±144	61.4±6.54

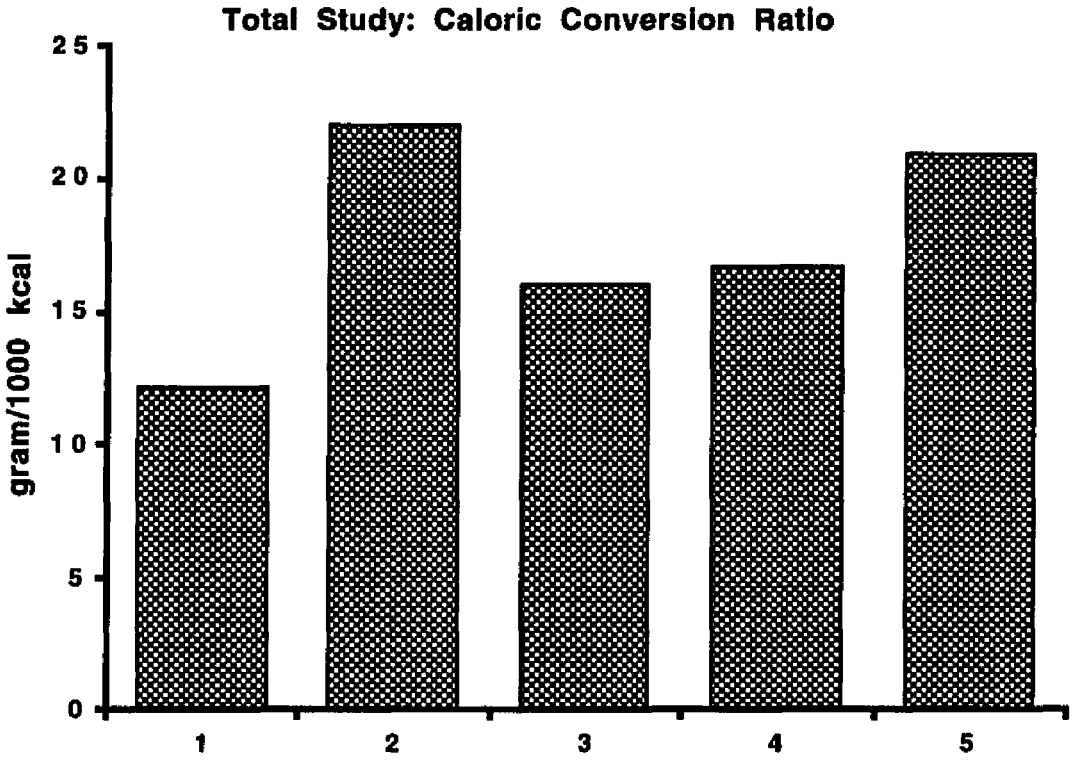

Figure 12.0 Total Study: Caloric Conversion Ratio
Treatment: 1. Sham (12), 2. No hormone (21) 3. Estrogen (15), 4. Estrogen and Progesterone(16), 5. Progesterone (20). 2-5 were ovariectomized.

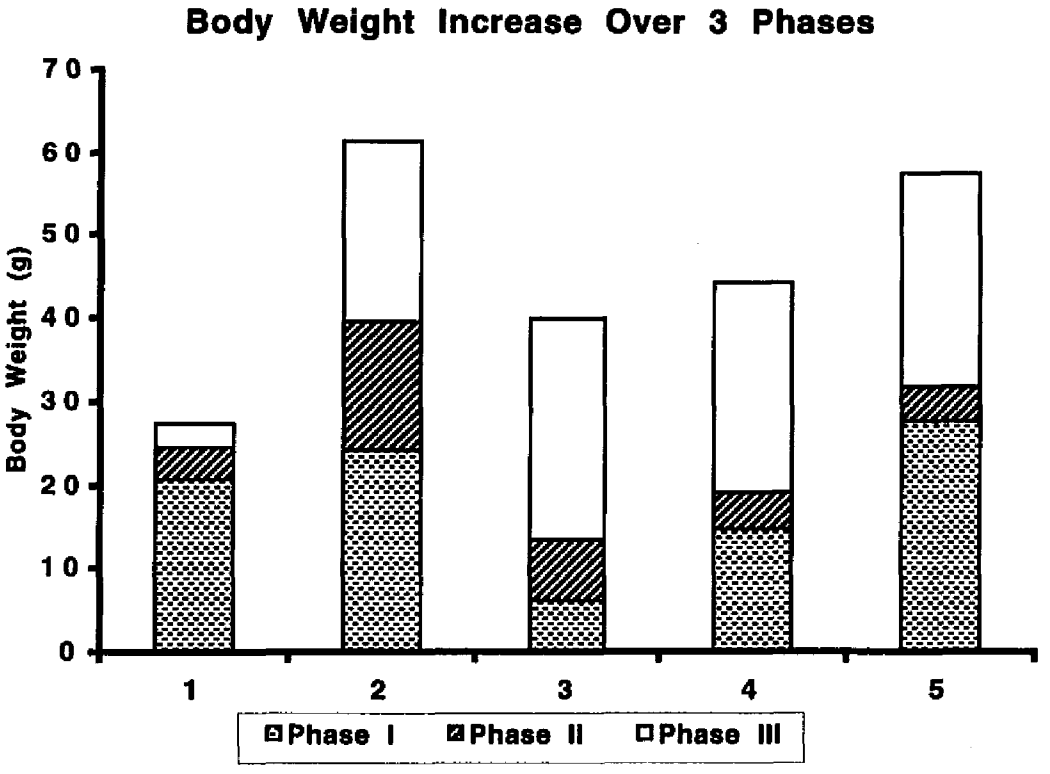

Figure 13.0 Body weight increases over three phases
Treatment: 1 Sham, 2. No hormone, 3. Estrogen, 4. Estrogen and Progesterone, 5. Progesterone, 2-5 were ovariectomized.

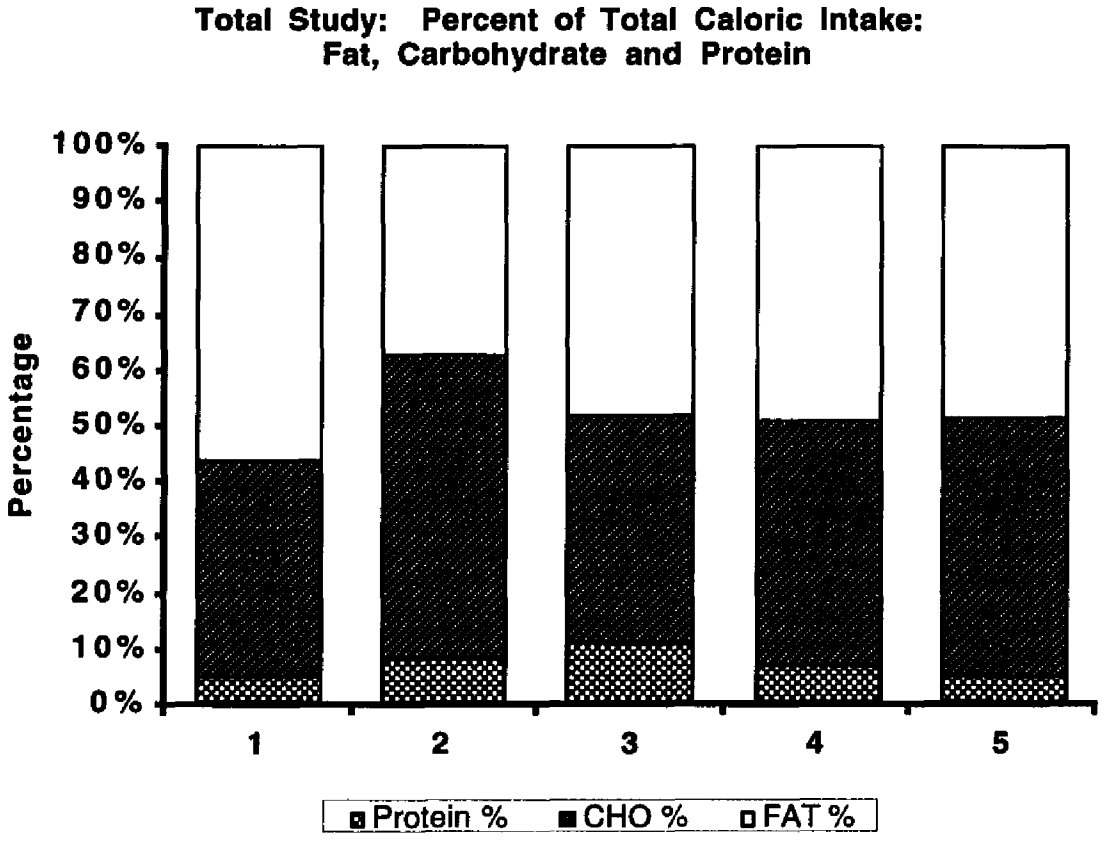

Figure 14.0 Total Study: Percent of total caloric intake: fat, carbohydrate, and protein
Treatment: 1. Sham, 2. No hormone, 3. Estrogen, 4. Estrogen and Progesterone, 5. Progesterone. 2-5 were ovariectomized.

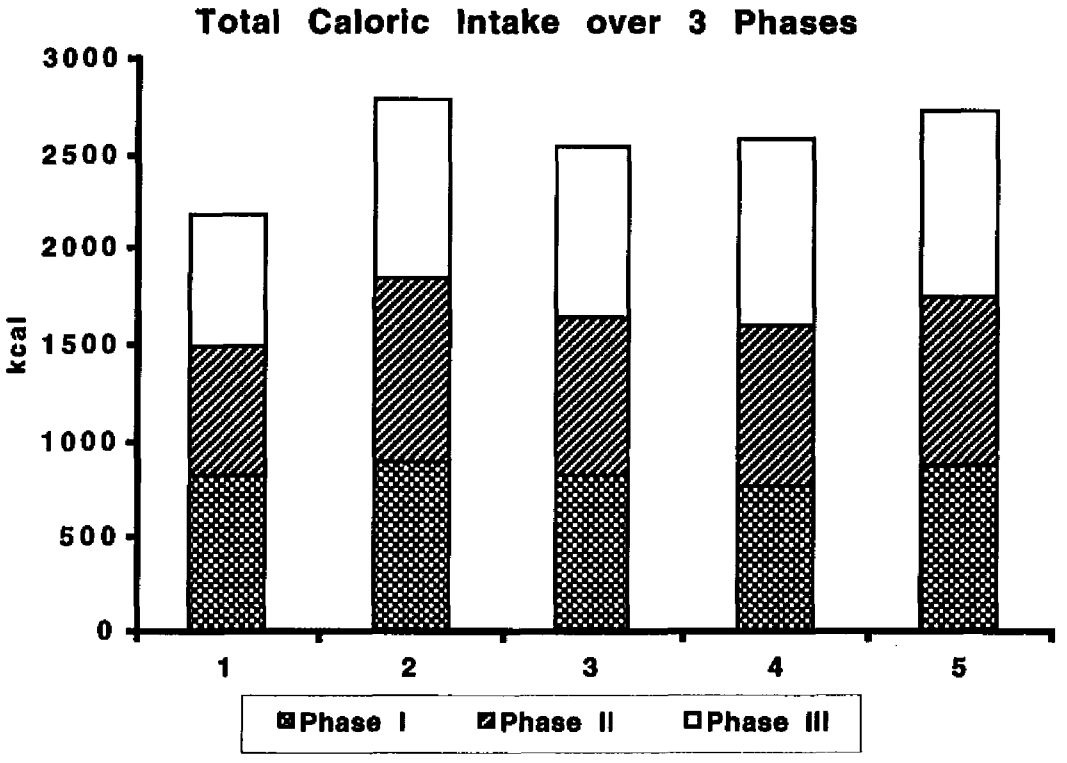

Figure 15.0 Total caloric intake over 3 phases
Treatment: 1 Sham, 2. No hormone, 3. Estrogen, 4. Estrogen and Progesterone, 5. Progesterone, 2-5 were ovariectomized.

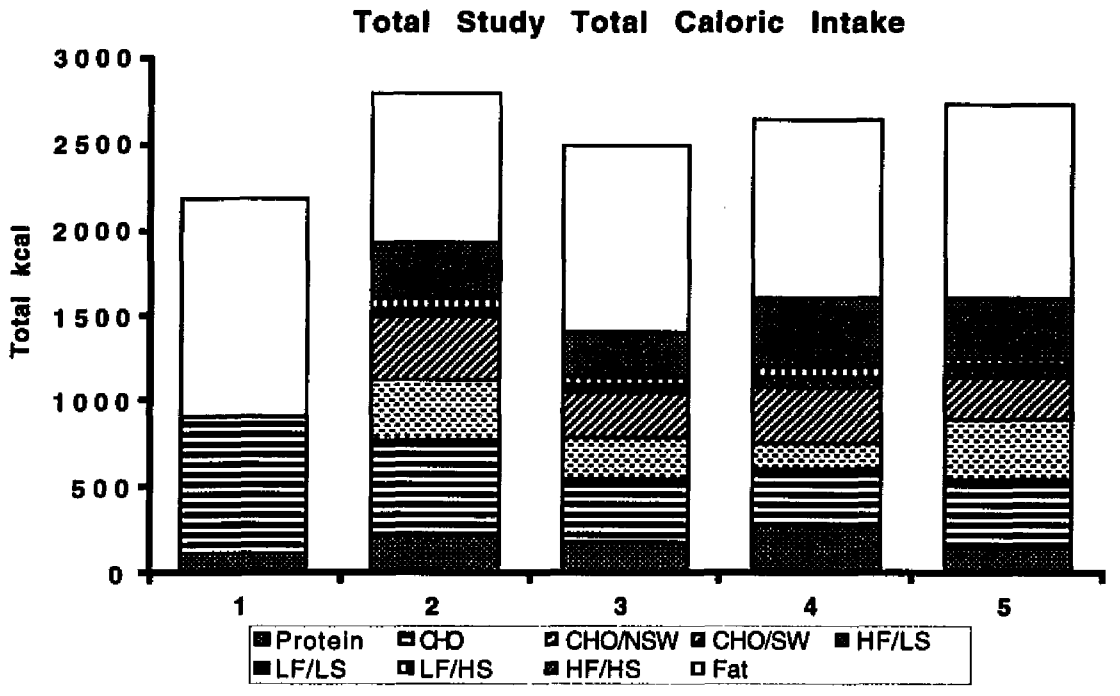

Figure 16.0 Total Study Total Caloric Intake
Treatment: 1 Sham, 2. No hormone, 3. Estrogen, 4. Estrogen and Progesterone, 5. Progesterone, 2-5 were ovariectomized. Macronutrient profile for macronutrients and chocolate choices.

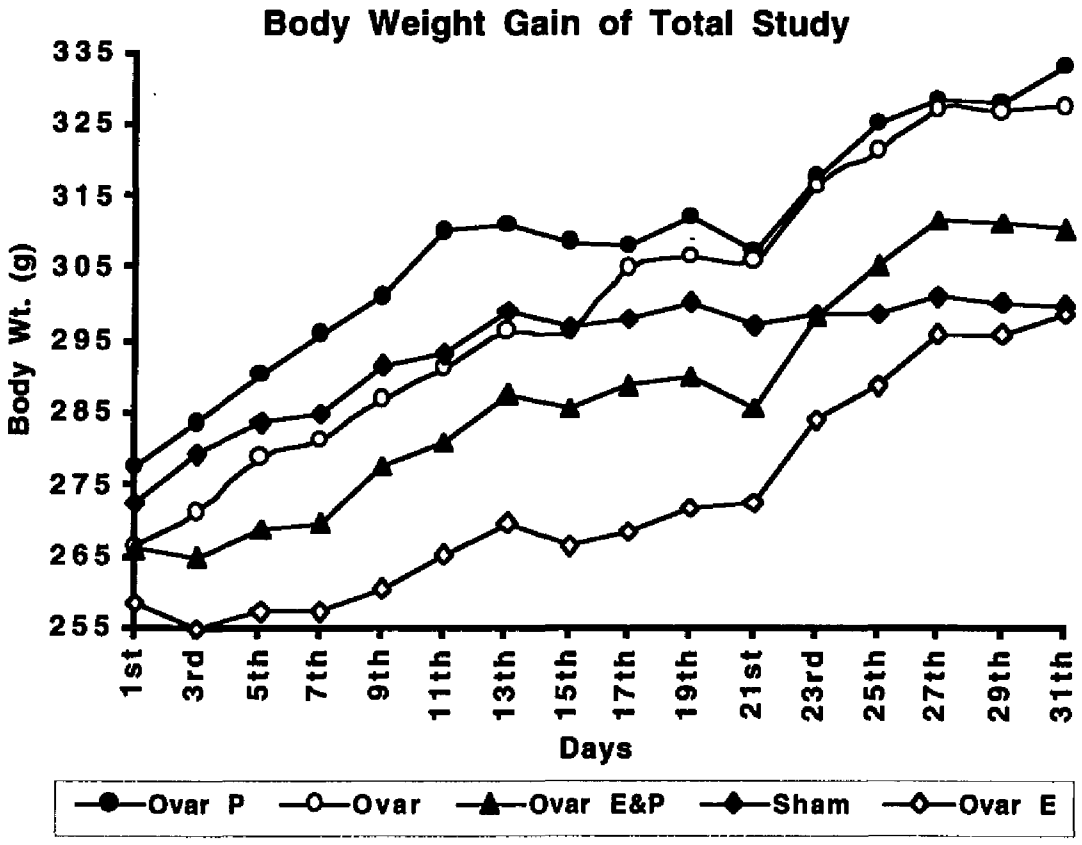

Figure 17.0 Body weight gain of total study
Line graph of variation of 30 day, body weight gain. Phase I data starts on day I, Phase II data starts on 11, Phase III data starts on day 21.

VITA

Jan Hamilton enjoyed science from early years and was always curious about why things happen. She grew up on a West Texas ranch on land which was claimed by her great grandfather in the 1800s. Cattle and 4-H programs were a happy part of her childhood. She met personally with three U.S. Presidents as a result of leadership and citizenship awards. In 1960 she entered Texas Tech University to study Nutrition. She graduated in three years so that she could work and put her husband through law school. There were five in his family in university, he had just finished a B.S. in Engineering, and did not feel he could be a further drain on his family. Tom has been extremely supportive of Jan in the pursuit of her Ph.D. They have three sons, John is an engineer, Brent is in law school and Rob is studying economics at Texas Tech University. The whole family enjoys weekends at the ranch.

During past years Jan worked as a dietitian and nutritional consultant both in hospitals and industry. She returned to Texas Tech University in 1987 to pursue a Ph.D in nutrition to qualify her for international nutritional consulting opportunities. A hormone project in Biochemistry at the Texas Tech University School of Medicine stimulated interest in the current research topic and motivated Jan to make every effort to become eligible for further research involving eating behavior, health parameters and states of disease. To earn the Ph.D at another university would greatly enhance her chance for future employment there. Therefore, she chose LSU with the Pennington Biomedical Center to finish the Ph.D., and to continue to work in her chosen field of Physiological Nutrition, Biochemistry and Ingestive Behavior.

DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Jan B. Hamilton

Major Field: Human Ecology

Title of Dissertation: Effects of Exogenous Female Sex Hormones on Food Intake, Macronutrients and Body Weight in the Ovariectomized Postbreeder Female Rat

Approved:

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

Albert H. Meier

J. Samuel Godber

Billie Collier

Michael Keenan

Date of Examination:

March 16,1995