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The Effects of Hypoestrogenism on Rest and Acute Submaximal Exercise Metabolism Using a GNRH Agonist Analogue Model.

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THE EFFECTS OF HYPOESTROGENISM ON REST AND ACUTE SUBMAXIMAL EXERCISE METABOLISM USING A GNRH AGONIST ANALOGUE MODEL

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
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Doctor of Philosophy

in

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by
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Grátias agámus Dómino Deo nostro, dignum et justum est.

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ABSTRACT

During menopause, many women experience weight gain which is mostly in the form of abdominal and upper body fat deposition. This phenomenon has been recognized as an independent predictor of cardiovascular disease in females. Other disease processes, such as diabetes, hypertension and some cancers, specifically, breast and esophageal have been linked to obesity. Thus, the occurrence of increased body fat is placing the postmenopausal female in jeopardy of a potential life-threatening position. The connection between the lack of estrogen seen in menopause and weight gain remains unclear, but studies have shown that estrogen status may affect carbohydrate and fat metabolism. This study examined the metabolic responses at rest and during a low-to-moderate intensity cycle ergometer exercise session of thirty-two premenopausal females at different levels of estrogen status. Eleven subjects (Group M) were tested during day 1 thru 4 of their menses which represented low estrogen status; fourteen subjects (Group F) were tested during the follicular phase (day 8 thru 12) which represented high estrogen status; and 7 subjects (Group G) who were being treated with a GnRH agonist analogue (Depot Lupron) to suppress estrogen synthesis, were tested to represent the post-menopausal female. Estrogen status was confirmed by blood analysis for all subjects.

Testing consisted of a 15-minute rest period and a 20 minute submaximal cycle ergometer test. The exercise protocol consisted of 2 minute staged increments of 10 watts, beginning at 0 watts, until the subject reached between 60 and 70% of her age-predicted maximum heart rate (220 minus age). Respiratory

gases were analyzed using the Quinton QMC™ metabolic cart in the breath-by-breath mode. Blood was drawn at the end of the rest session, at 10 minutes and 20 minutes of exercise.

Results indicated that the pseudomenopausal model demonstrated non-significant differences in fuel substrate utilization. It is possible that the lack of estrogen is not an underlying factor of the weight gain seen in menopause, but further investigation is required.

CHAPTER 1. INTRODUCTION

Many women experience weight gain at the time of menopause. Some investigators have found that the body mass index (BMI) is significantly higher than the ideal value in climacteric (the transition from late fertile age to advanced natural post-menopause) women, and that menopause seems to be a weight-gain inducing co-factor (de Aloysio, *et al.*, 1987). It has been suggested that postmenopausal females may be heavier than their younger premenopausal counterparts because of obscure reasons attributed to the aging process and changes in lifestyle (Gaspard, 1994). This includes a general decrease in activity levels which may be related to aging. Without a concomitant decrease in energy intake, this factor could lead to increased storage of energy as fat. Moreover, if activity decreases, it is probable that musculature also decreases accordingly. Since basal and resting metabolic rates (minimum energy expenditure required to maintain bodily cellular functions) are directly related to the amount of lean, metabolically active, tissue (Shutz, 1993), it follows that basal and resting energy expenditure will decrease. This could be a valid explanation for weight gain if dietary habits are unchanged. Another plausible connection to the weight gain may be an increased consumption of calories due to increased appetite, which, if more than calorie expenditure, ultimately leads to increased weight in the form of fat. In addition, aging has been shown to be associated with detrimental changes in glucose tolerance and subsequent weight gain, but training studies involving the elderly described a reversal of these changes, thus indicating that lifestyle may be more of a factor than aging *per se* (Davidson, 1979; DeFronzo, 1979).

While all of the above reasons may offer insight into postmenopausal weight gain, there may also be very important, although subtle, metabolic changes that develop concurrently with the hormonal alterations that occur during the menopause that are not age-related. Even though training has been shown to eliminate the decremental effects of aging as cited above, many studies have shown that menopause has an appreciable effect on insulin and glucose metabolism (Gaspard, 1994; Stevenson, *et al.*, 1994) as well as lipid metabolism (Jensen, 1994). The major hormonal alteration is decreased estrogen synthesis that results from natural ovarian failure that occurs during menopause. Senoz and associates (1996) found that estrogen deprivation rather than age, is responsible for the poor lipid profile and carbohydrate metabolism in postmenopausal women. These effects could alter the body's use of fuel substrates, both in exercise as well as resting metabolism, and could adversely affect body fat weight.

If postmenopausal weight gain is caused by alterations in metabolism, it is reasonable to suspect that the body prefers to use carbohydrate, thus sparing fat and directing it to storage in fat cells (adipocytes). The amount of carbohydrate and fat being used as a fuel substrate (oxidized) may be quantified by a method termed indirect calorimetry. This method of ascertaining fuel substrate utilization requires the measurement of oxygen consumption and carbon dioxide production, the ratio of which (VCO_2/VO_2) is called the respiratory quotient (RQ or R), and is described in the Methods section of Chapter 3.

The predominant fuel source at rest is lipid and the R value is close to 0.70. As intensity of exercise increases, carbohydrate becomes more important, and the

R value approaches unity. There is a mid-point where half the fuel being oxidized is carbohydrate and the other half is lipid. This has been termed the "crossover point" and is the power output at which energy from carbohydrate predominates over energy from lipid, with further increases in power eliciting a relative increment in carbohydrate utilization and concomitant decrement in lipid oxidation (Brooks & Mercier, 1994).

It is apparent that age is an unavoidable confounding factor when studying the postmenopausal female. An alternative, therefore, is to use a psudomenopausal model which eliminates the factor of age. Such a model is represented by female patients who are being treated for endometriosis with a GnRH agonist analogue (GnRHaa) such as leuprolide acetate (Lupron Depot; TAP Pharmaceuticals, Deerfield, IL). Levels of circulating estrogens are suppressed to menopausal range within 4 weeks of initiation of this therapy (Surrey, *et al.*, 1995). This group can then be compared to premenopausal, eumenorrheic females during menses and follicular phases of their cycles, representing a range of estrogen levels.

The purpose of this study was to determine the influence of estrogen levels, independent of age, on physiological, metabolic parameters at rest and during submaximal exercise that would indicate a rationale for increasing fat weight. The criterion dependent variable under study was the R value. Changes in the following factors were also determined in order to obtain a profile of the *in vivo* environment at various levels of estrogen within different levels of energy expenditure: blood levels of cortisol, glucose, lactate, ammonia, hematocrit;

cardiorespiratory factors of heart rate and percent of age-predicted maximum heart rate; and metabolic factors of percent of fuel substrate utilization obtained from carbohydrate sources, resting energy expenditure (kilocalories expended per day), kilocalories expended per liter of oxygen and kilocalories expended per minute.

It was hypothesized that hypoestrogenism may result in higher R values during rest and submaximal exercise at various absolute and relative workloads thus representing an overall preference for carbohydrate, leading to a shift in the proportion of substrate oxidized. Moreover, if carbohydrate is the preferred fuel substrate, and normal fat metabolism suffers, it may explain why the body will store the excess calories in adipose tissue. It was further hypothesized that if the postmenopausal female is preferring carbohydrate to fat, the crossover point will be attained sooner (at a lower power output). It was assumed that protein utilization is minimal (5 - 10%) and is equal among untrained individuals irrespective of menopausal status (See Chapter 2 for review).

It is recognized that the results of this study will be limited to the population from which data was gathered. Specifically, the subjects under study were healthy, premenopausal females between the ages of 21 and 42 who were not at the "athletic" level of fitness, and ranged from inactive to moderately active.

CHAPTER 2. REVIEW OF LITERATURE

Estrogen levels normally decline at menopause. Since estrogen deficiency occurs at menopause, and increased body weight may be demonstrated at this time, it is easy to observe a link between hypoestrogenism and weight gain. However, this could be a simple coincidence, with no "cause" of weight gain being attributed to the lack of estrogen.

Common menopausal symptoms of hot flushes, depression, changes in balance and limb coordination, and decreased alertness have been shown to be attenuated or completely alleviated using estrogen replacement therapy (ERT) (Backstrom, 1995). Research trials using ovariectomized animal models have demonstrated that weight gain is also diminished with the administration of high doses of estrogen (Kendrick, *et al.*, 1987; Perry, *et al.*, 1979; Wade, 1975). Even though the mechanism of action is not clear, estrogen may have an effect on how the body uses energy. The present study investigated the differences between pre- and postmenopausal use of energy for the purpose of identifying metabolic reasons for weight gain commonly seen in the postmenopausal years.

The Biochemistry and Synthesis of Estrogen

There are basically two categories of hormones produced by the body, steroid and nonsteroid. Nonsteroid hormones include amino acid derivatives, peptides, glycoproteins and eicosanoids. Steroid hormones are cyclic hydrocarbon derivatives of cholesterol and include progestins, androgens, corticosteroids and estrogens. Estrogens are a family of female sex hormones which are synthesized primarily in the ovaries of adult women before the onset of

menopause. The estrogen molecule has a four ring aromatic structure derived from cholesterol. Its synthesis begins with cholesterol and proceeds through several stages of reactions before reaching the final product of estrogen. Essentially, the 21-carbon cholesterol molecule is cleaved of its carbon chain, is hydroxylated, reduced, oxidized, isomerized, and finally, aromatized to form an 18-carbon estrogen molecule. Highly specific enzymes, as well as NADPH and O_2 are required in these reactions. A simplified flow of estrogen synthesis from cholesterol is shown in Figure 2.1.

Although six different natural estrogens have been isolated from the human female, only three forms (estradiol, estrone, and estriole) are present in significant quantities (*Facts and Comparisons*, 1995). Their differences are found on carbons C_{16} and C_{17} of the D ring. The strengths of their action are also different. Estradiol has 12 times the estrogenic potency of estrone, and 80 times that of estriole (Guyton, 1986).

Estradiol, also termed E_2, 17β-hydroxysteroid, or estradiol-17β, is not only the most potent estrogen, but is the most predominant form produced by the body. The terms "estrogen" and "estradiol" are, therefore, used interchangeably. Premenopausal blood levels of estradiol depend on the day of the menstrual cycle, the approximate range being 100 - 700 pg/ml (Guyton, 1986). Blood estradiol concentrations are lowest during menses, rise during the proliferative, follicular phase, and peak before ovulation (Figures 2.2 and 2.3). The level

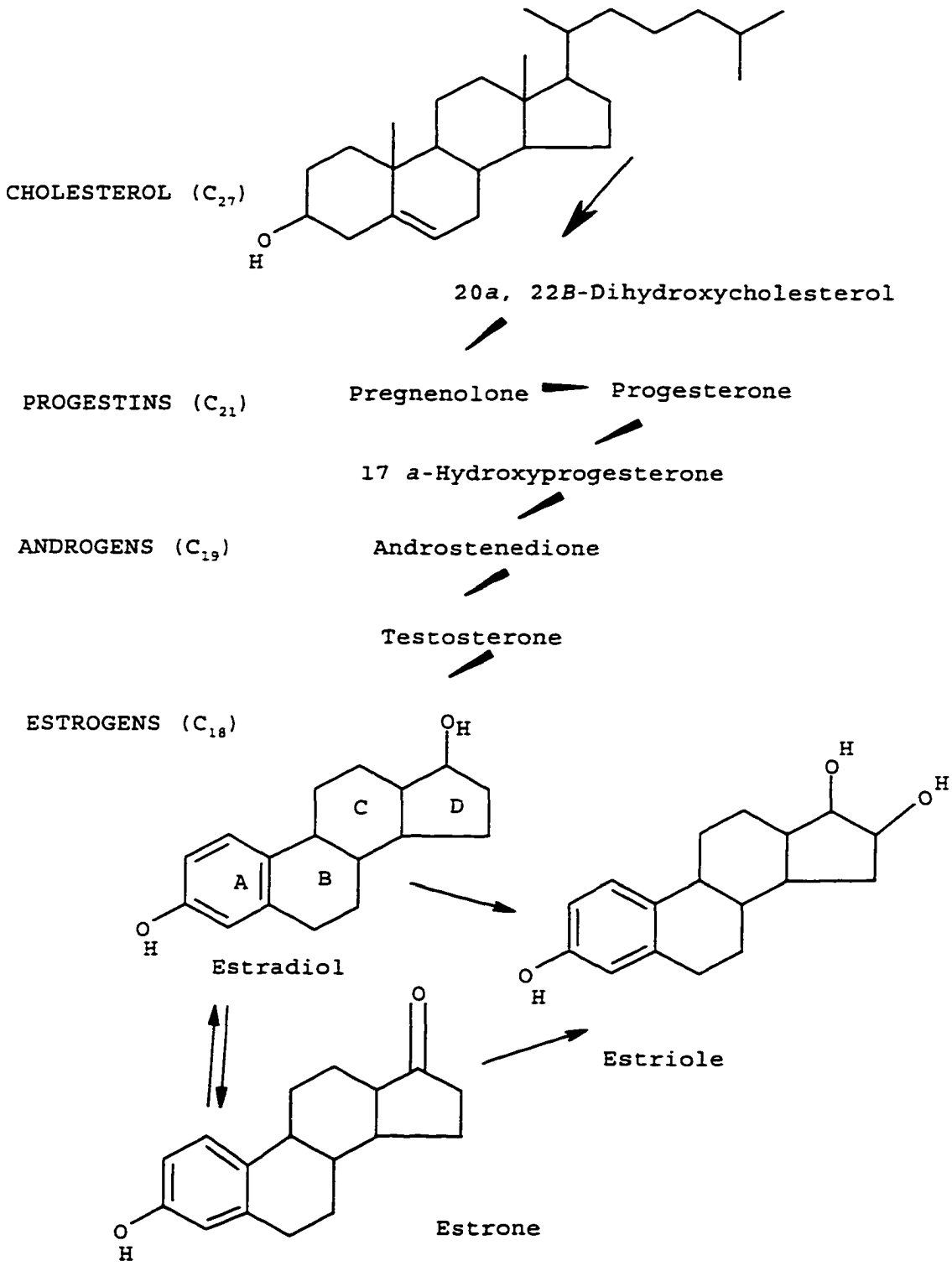

Figure 2.1. Synthesis of Estrogens from Cholesterol

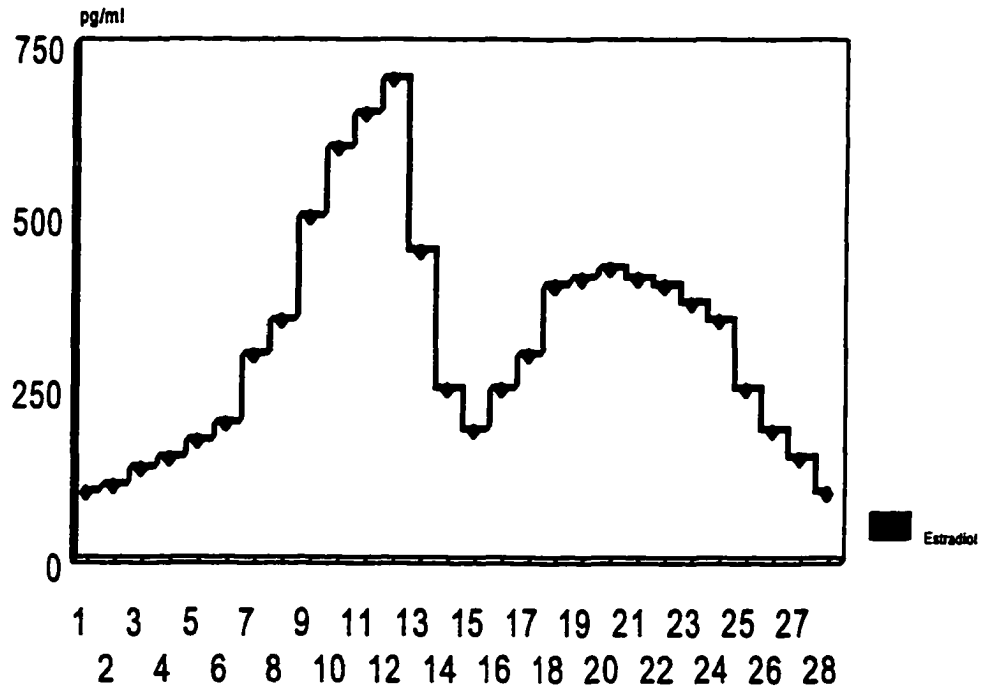

Figure 2.2. Approximate Plasma Concentrations of Estrogen in the Premenopausal Female During the Normal Sexual Cycle. Source: Adapted from Guyton, 1986.

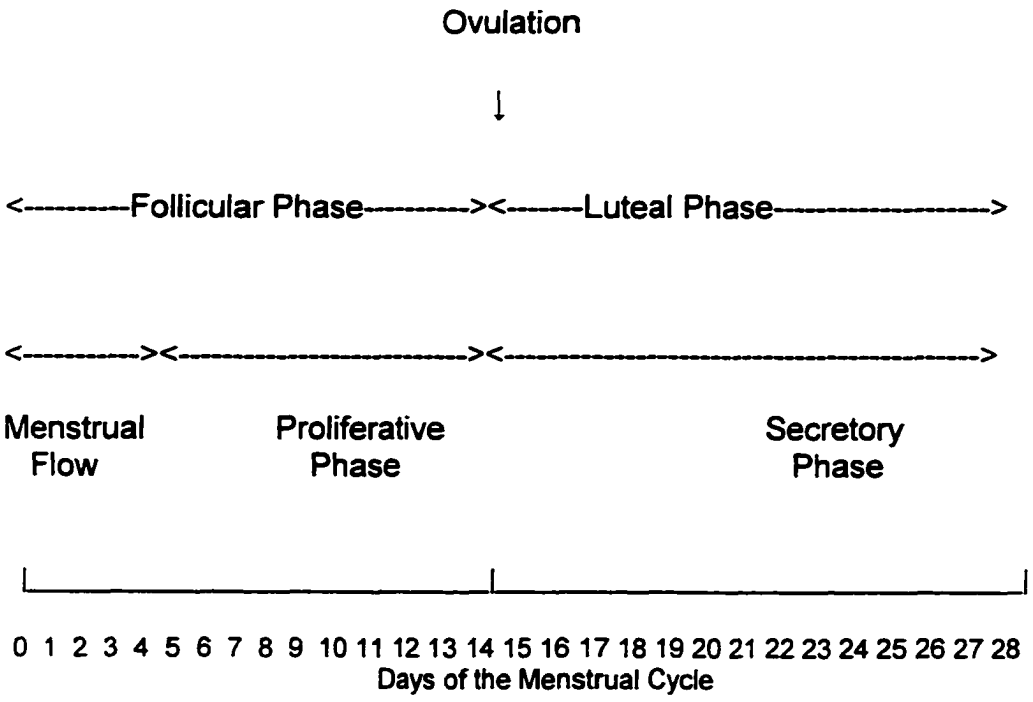

Figure 2.3. The Menstrual Cycle Phases

of estrogen that elicits menstrual bleeding and development of endometrium varies among individuals, but a continuous serum concentration of greater than 100 pmol/l will usually have these effects (Backstrom, 1995). Table 2.1 defines typical or "expected" values of estrogen at different phases in the menstrual cycle and compares them with postmenopausal values and those of males.

Estradiol is rapidly oxidized to a weaker estrogen form, estrone. Small amounts of estrone are secreted by the thecal and stromal cells of the ovaries (Guyton, 1986) of the premenopausal female, but most estrone is synthesized in the peripheral tissues from androgens secreted by the adrenal gland. Estrone and estradiol are interconverted by the enzyme estradiol-17B-hydroxysteroid dehydrogenase (Bayard, *et al.*, 1995) which favors estrone production. Estriole, the weakest form of estrogen, is an oxidative product derived from either estradiol or estrone, and this conversion occurs mainly in the liver (Guyton, 1986). Ultimately, estrogens are conjugated in the liver to form glucuronides and sulfates which are excreted in the urine and bile.

The postmenopausal range of serum E_2 is less than 125 pmol/l (Matta, *et al.*, 1988) or 25.0 ± 8.0 pg/ml (Dumesic & Matteri, 1993) and a mean postmenopausal value has been found to be 0.03 (30 pmol/l)± 0.01 nmol/L (Rebuffe-Scrive, *et al.*, 1986). Ovarian steroidogenesis contributes little to this circulating pool (Judd, *et al.*, 1976). After the onset of menopause, the ovaries usually produce very little estrogen, but continue to produce androgens, especially testosterone and androstenedione, by the ovarian stromal and hilus cells which

Table 2.1. Summary of Expected Estradiol Values

Estrogen Status of:		n	Central 95th Percentile (pg/ml)	Entire Range (pg/ml)
Normally Menstru-ating Females	Follicular Phase	178	25* - 145	25* - 221
	Mid-Cycle Peak	150	112 - 443	83 - 690
	Luteal Phase	210	48 - 241	26 - 408
Postmenopausal Females		71	25* - 59	25* - 86
Males		103	25* - 50	25* - 70

*Values of less than the sensitivity of the assay reported as 25 pg/ml.
Source: Adapted from Abbott Laboratories Diagnostics Division, Abbott Park, IL, IM®x Assay Insert.

retain LH receptors (Judd, *et al.*, 1974). Androstenedione is also produced by the adrenals. The androgens produced by the ovaries and adrenals are aromatized mainly to the estrone form of estrogen (Grodin, *et al.*, 1973). Extragonadal aromatization sites that have thus far been identified, other than the adrenals, are adipose tissue, liver, kidney and brain and mainly involves the conversion of androstenedione to estrone (Grodin, *et al.*, 1973) . Most of the postmenopausal estrogen metabolism occurs in adipose tissue (Dumesci & Matteri, 1993). Vascular endothelial cells have also been shown to synthesize estrogens. These cells also contain functional estrogen receptors (Bayard, *et al.*, 1995). Age and weight both influence extragonadal aromatization as the production of androgens by the adrenals declines by the age of 30 (Backstrom, 1995). Heavier women have higher conversion rates and higher plasma estrogen levels than thinner women which has been shown by a close relationship between the body mass index and estrogen levels (Boman, *et al.*, 1990).

The Mechanism of Action of Estrogen

As described above, estrogen is produced in the ovaries, secreted into the bloodstream, and taken up by most tissues, but mainly those of sex organs and adipose tissue. Estrogen's actions are varied. It is responsible for the synthesis of structural proteins for tissue growth, repair and maintenance of female sex organs, and is, therefore, anabolic. It also promotes the development of female sexual characteristics, including fat deposition; assists in regulating the menstrual cycle, specifically, the proliferative phase of the menstrual cycle; and participates in oogenesis, ovulation, and many changes during pregnancy. In

addition, estrogen indirectly contributes to the integrity of the skeleton by conserving calcium and phosphorus and encouraging bone formation (*Facts and Comparisons*, 1995).

Estrogen is lipid soluble and can, therefore, pass easily through the target cell's membrane into the cytosol where the specific estrogen receptors are found. Its primary effects, like all steroid hormones, are on gene expression rather than on enzyme activities or transport processes (Stryer, 1988). Hence, the newly formed hormone-receptor complex enters the nucleus of the cell, binds to a particular enhancer section of the cell's DNA, and activates certain genes. In response to this direct activation, mRNA is synthesized and exits the nucleus into the cytoplasm, where it promotes protein synthesis.

Even though estrogen has a wide variety of effects on different organs, estrogen receptors are encoded by a single gene and are members of the steroid/thyroid hormone receptor superfamily which also include receptors for active metabolites of vitamins A and D (Evans, 1988). To achieve its diversity of action, it is postulated that a second mediator of estrogen action, the estrogen receptor-regulated transcription factor which amplifies the action of estrogen, forms a cascade of gene regulation to provide diverse and specific pathways in each target organ (Muramatsu, 1995).

Recently, estrogen receptors have also been found on the plasma membranes of cells of the endometrium, liver, pituitary, and other tissues (Baulieu & Robel, 1995). This type of hormone-receptor complex causes more of a short-term effect, *i.e.*, within minutes, compared to the intracellular hormone-receptor

complex, *i.e.*, hours, and it is not known what effect, if any, is being demonstrated.

Estrogen Regulation and Control

Female sex hormones are intricately regulated by negative and positive feedback as well as neural mechanisms and depend on menstrual cycle phases (See Figure 2.4). There are at least three separate organs which are important in the regulation of estrogen synthesis and release: (1) the hypothalamus, (2) the adenohypophysis (anterior pituitary), and (3) the ovaries.

The hypothalamus synthesizes and releases two gonadotropin releasing hormones, follicle-stimulating hormone releasing hormone (FSHRH) and luteinizing hormone releasing hormones (LHRH) in a pulsatile fashion. Collectively, these two hormones are called gonadotropin releasing hormones, or GnRH. They are believed to be part of one hormone complex with different active sites which are specific to their particular receptors. The release of GnRH is modulated by both negative and positive feedback mechanisms: It is directed by both neural input and circulating levels of the hormones, progesterone and estrogen, FSH and LH, as well as levels of GnRH.

The anterior pituitary has specific receptors for both FSH- and LH-releasing hormones. The hormone-receptor complex initiates and stimulates the pituitary to release one or both of the gonadotropins, FSH and LH. Generally, these two hormones are responsible for the cyclic nature of ovulation and the menses, as well as of the secretion of estrogen and progesterone by the ovaries. Normal

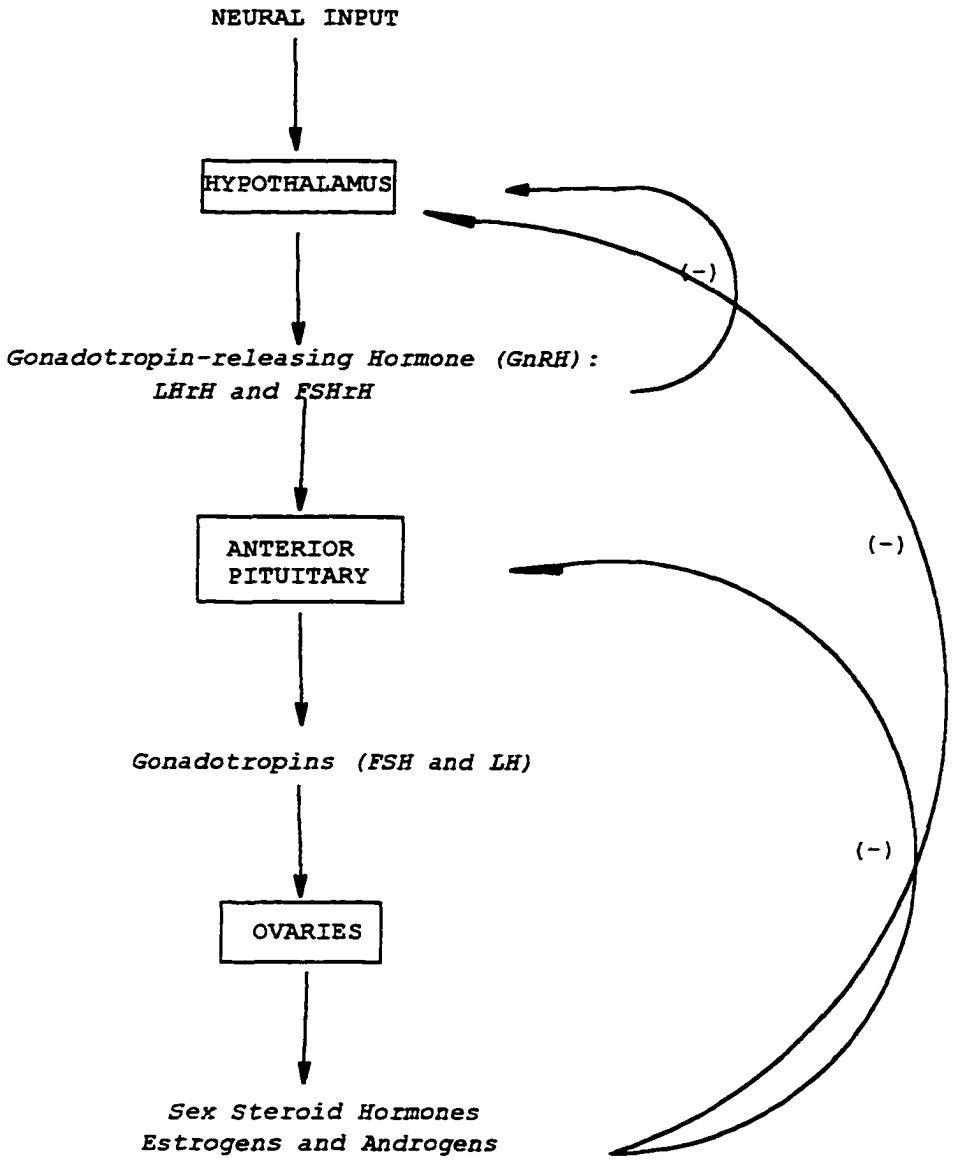

Figure 2.4. Estrogen Regulation and Control

plasma levels are also cyclic. Ovaries which are not stimulated by gonadotropic hormones remain completely inactive (Guyton, 1986).

When FSH and LH reach the ovaries, they attach to their specific receptors in cell membranes. Estrogen synthesis and secretion is dependent on the activity of FSH and LH acting on the maturing follicle and corpus luteum during the menstrual cycle. Some estrogen is secreted by the corpus luteum during the luteal phase of the menstrual cycle, after ovulation, but most of the estrogen is secreted by the ovarian follicle before ovulation.

Hormonal Changes at Menopause

At the time of menopause, the hormone milieu changes. The ovary no longer contains viable primordial follicles, and therefore does not depend on the gonadotropins to nurture the follicle's development, hence, ovulation does not take place. The major consequence is cessation of ovarian estrogen production. Since follicular estrogen production is diminished, the hypothalamus only receives negative feedback of decreased estrogen status. Therefore, the hypothalamus continues to secrete GnRH, and the pituitary continues to respond with a higher than normal FSH and LH release. In fact, an increase in early follicular and mid-cycle levels of FSH is a marker of the aging ovary (Sherman, *et al*, 1976).

Acute Responses of Estrogen to Exercise

Changes in plasma levels of estrogen during exercise have been found to depend on the phase of the menstrual cycle, the training status of the individual, and the intensity of exercise. During the luteal phase, estrogen levels have been shown to rise with intensity and to level off during exhaustive exercise (Figure

2.5). In the follicular phase, however, exercise-induced increases in estrogen have been shown to be significant only at exhaustion (Jurkowski, *et al.*, 1978). During the menses, no changes have been observed in estrogen concentrations during thirty minutes of exercise at an intensity of 74% VO_2max (Bonen, *et al.*, 1979). There is also a demonstrated difference in estrogen response due to training status of premenopausal women. Bonen and associates (1979) showed that heavy exercise in untrained subjects provoked a significant increase in estrogen levels, whereas trained subjects did not elicit an estrogen response at the same absolute workload.

Changes in Fuel Substrate Metabolism at Menopause

Even though carbohydrate is actually preferred, especially at higher intensities of exercise, fat is readily used to preserve carbohydrate stores as well as protein stores, *i.e.*, lean tissue, or lean body mass, at lower intensities. Therefore, fatty acids are the main fuel substrates used at rest and during submaximal (light) activities. They are derived from circulating blood lipids, from lipolysis of triglycerides in adipocytes, and from lipoprotein lipase (LPL) activity in fat cells stored in muscle. Because of the abundance of adipose tissue in t h e body, this fuel substrate is not thought to be a limiting factor in endurance activities.

Adipose tissue is responsive to lipolytic stimuli such as hormonal input, *i.e.*, glucagon, catecholamines, growth hormone, cortisol. The response varies, however, depending on the location of the adipose tissue. For instance, femoral adipose tissue has been shown to be less responsive to lipolytic stimuli than

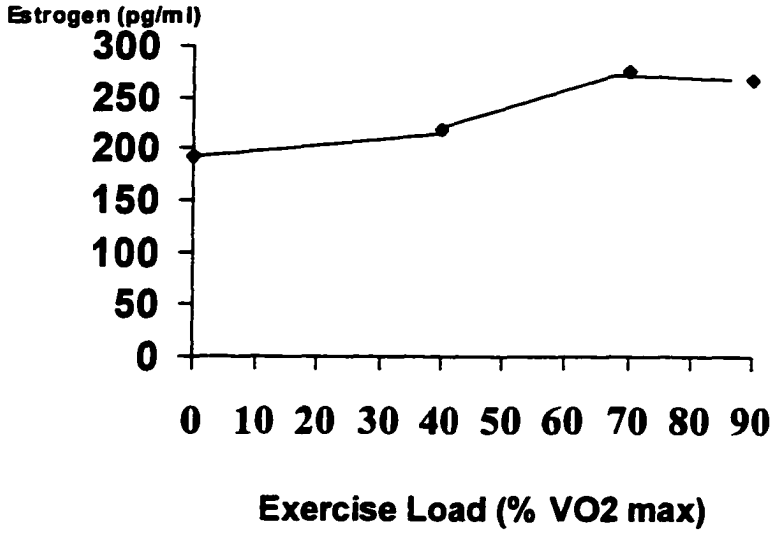

Figure 2.5. Responses of estrogen to various exercise intensities during the luteal phase of the menstrual cycle. (Based on data from Jurkowski, *et al.*, 1978.)

abdominal adipose tissue (Kather, *et al.*, 1977; La Fontan, *et al.*, 1978; Rebuffe-Scrive, *et al.*, 1985; and Smith, *et al.*, 1979). However, lipoprotein lipase (LPL) activity has been shown to be highest in the femoral region (Guy-Grand & Rebuffe-Scrive, 1980; Lithell & Boberg, 1978). A comparative study performed by Rebuffe-Scrive and associates (1986), demonstrated that femoral adipocytes of premenopausal women had a higher LPL activity than their abdominal or mammary adipocytes. Postmenopausal females revealed no regional differences in LPL activity. There was a trend for the premenopausal females to have a higher LPL activity in the femoral region than the postmenopausal females. In response to norepinephrine stimulation, premenopausal females demonstrated lower lipolysis in the femoral region than the other regions, and lipolysis was slightly higher in the mammary than in the femoral and abdominal regions of the postmenopausal females. However, both mammary and abdominal lipolysis was lower in the postmenopausal females as compared to the premenopausal females (lipolysis of the femoral region was unchanged after menopause). From these results, it was hypothesized that adipose tissue distribution may be caused by regionally specific effects of sex steroid hormones on adipocyte metabolism. Furthermore, other investigators have reported an increased resting level of plasma free fatty acids in postmenopausal women (Morrow, *et al.*, 1981), indicating either increased mobilization of adipose tissue triglyceride fatty acid stores or decreased free fatty acid clearance (Jensen, *et al.*, 1994).

Results of animal studies have also indicated that estrogen replacement in oophorectomized rats influenced myocardial glycogen utilization during exhaustive

exercise, with a dose response relationship, and spared tissue glycogen during submaximal exercise (Kendrick, *et al.*, 1987). If hypoestrogenemia results in a lower initial glycogen level at the onset of exercise, due to the body's preference for glucose over fat at rest, it would follow that endurance exercise performance would be adversely affected.

Protein contribution to energy production may be relatively small but not insignificant. When needed for fuel, endogenous and exogenous proteins are catabolized to their amino acid building blocks and used as oxidative substrates and as gluconeogenic precursors (Dohm, *et al.*, 1982, 1985, 1987). Several amino acids provide the carbon sources for gluconeogenesis during exercise and glycogen restitution during recovery, and others participate in important anaplerotic functions (Brooks, 1987). Table 2.2 displays the percentage of fuel as protein found by Henderson and associates (1985). These percentages were computed from mean data of leucine oxidation and oxygen consumption of treadmill run rats. Some investigators have estimated 5 -10% of the fuel for prolonged exercise is provided by amino acids (Lemon & Mullin, 1980). It is well established that leucine metabolism is affected by acute aerobic exercise and that it is a primary amino acid source of oxidative energy (Hagg, *et al.*, 1982; White & Brooks, 1981; Wolfe, *et al.*, 1982). The adrenal cortex hormone, cortisol, exhibits the most potential for inducing heightened protein catabolism (Long & Lowry, 1990) in order to assist in maintaining blood glucose homeostasis (Brooks & Fahey, 1985). As a "stress" hormone cortisol is released as a result of both physical and mental stressors, and increases the catabolic breakdown of proteins and the

Table 2.2. Percentage of Energy Derived from Protein.

	Untrained	Trained
Rest	5.0	7.1
Low Intensity Exercise	4.2	6.0
Moderate Intensity Exercise	(Not reported)	6.2
SOURCE: Adapted from Henderson, *et al.*, 1985		

formation of glucose, which in turn may enter the metabolic pool (Åstrand & Rodahl, 1986). Blood cortisol levels, at rest, follow a circadian rhythm pattern and vary considerably during the day. Furthermore, they increase during exercise at a rate proportional to intensity (Brooks & Fahey, 1985).

Rationale for Using the GnRHaa Model

Since menopause occurs at an average age of 49-50 years (Leidy, 1996), some of the demonstrated weight gain may be unspecified effects of age. In order to eliminate this possible confounding factor of age, a model has been chosen which represents the postmenopausal female, but is of premenopausal age. The chosen model is a patient receiving a gonadotropic releasing hormone agonist analogue (GnRHaa) for the treatment of endometriosis and in relieving endometriotic symptoms. This treatment modality has repeatedly been shown to induce a reversible pseudomenopausal state (Saltiel, 1991).

The decapeptide chemical structure of GnRH was discovered in 1971. Agonist analogues have been synthesized and successfully used in the treatment of endometriosis since 1981 (Wheeler, *et al.*, 1993). At the onset of administration, the GnRHaa stimulates the release of FSH and LH from the pituitary and results in an increase in estrogen synthesis. However, with repeated administration, the stimulatory effect is abolished and induces a down-regulation of the pituitary gonadotropin secretion. This results in a reversible state of oophorectomy with associated hypoestrogenic values equal to those found in the postmenopausal female (Johansen, *et al.*, 1988; Matta, *et al.*, 1988; Monroe, *et al.*, 1986; Schriock, *et al.*, 1985; Steingold, *et al.*, 1987). The diminished

circulating estrogen in turn causes the abnormal endometrial tissue to atrophy, thus eliminating the pain associated with endometriosis. GnRH and its analogues also exert direct effect on the ovary by binding to its specific receptor, stimulating inositol phospholipid (IP_3) turnover in the granulosa cells (Imai, *et al.*, 1993). The results can either be stimulatory or inhibitory, as above, depending on duration of treatment.

In clinical trials the GnRH agonist, depot leuprolide acetate (LA), has been shown to be effective in reducing estrogen concentrations to postmenopausal levels (Dumesic & Matteri, 1993; Dawood, *et al.*, 1995). After only one week of the administration of 3.75 mg depot LA, serum estradiol fell to postmenopausal range, 32.0 ± 4.4 pg/ml, and continued to fall to below 25.5 ± 5.0 pg/ml. Serum estradiol levels returned to a mean of 127.4 ± 37.3 pg/ml at 3 to 4 weeks after stopping the depot LA treatment (Dawood, *et al.*, 1995).

Major side effects of GnRHaa therapy are typical symptoms of menopause such as hot flashes and vaginal dryness (Matta, *et al.*, 1987). Less common side effects are weight gain, insomnia, decreased libido, and edema (Wheeler, *et al.*, 1993). Mineral bone loss has been reported after several months of GnRHaa administration (Dawood, *et al.*, 1995; Johansen, *et al.*, 1988; Kauppila,1993; Matta, *et al.*, 1987; Wheeler, *et al.*, 1993). Adverse changes in lipid profiles, *i.e.*, increased serum cholesterol and LDL cholesterol, have also been found after treatment (Johansen, *et al.*, 1988).

Summary

The above studies show evidence that the postmenopausal female may be favoring glucose over fat utilization at rest, as well as during low-moderate intensity exercise. If this is so, the postmenopausal female may store fat more easily than the premenopausal female and thus have a tendency toward the development of obesity. If lipid utilization is depressed, weight loss programs and exercise prescription methods should employ strategies that take this factor into consideration.

In summary, the GnRH model is being used to demonstrate the influence of diminished estrogen levels, experienced in menopause, on resting and moderate exercise metabolism. The model eliminates the influence of age on weight gain that may be experienced during menopausal years and is expected to be representative of the hormone milieu found postmenopausally.

CHAPTER 3. METHODS

Subjects

Thirty-two premenopausal women, aged 21 to 42 years (mean = 30.6) were recruited as volunteers to participate in one of three groups. Twenty-five of the women were eumenorrheic. Eleven were tested during their menses, day 1 - 4 (Group M) and fourteen were tested during the follicular phase of their cycle, day 8 - 12 (Group F). Seven of the women were being treated for endometriosis and had received at least two GnRHaa-depot injections (all were being treated with depot luprolide acetate). They were recruited from the pool of patients of local physicians, and were otherwise healthy (Group G). Estrogen status of each subject was confirmed by quantitaton of plasma estradiol levels during the fasting, resting phase before 8:00 a.m. on the day of testing. Inclusions to the program were those women who were

- Apparently healthy, with the exception of those who were being treated for endometriosis
- Between the ages of 21 and 42
- Untrained/sedentary to moderately trained (not participating in competitive athletic events)
- Not taking hormone replacement therapy or add-back therapy, or oral contraceptives
- Non-smokers
- Non-obese (Body Mass Index <28 kg/m^2)
- Non-hirsute

- Eumenorrheic (normal and regular menstrual periods for the past year, or amenorrheic secondary to GnRHaa therapy)

Exclusions included those women who had

- History of heart problems other than asymptomatic mitral valve prolapse
- Smoking history within the past year
- Drug therapy other than GnRH agonist
- Hypertensive history and/or resting systolic BP > 140
- Diabetic or diagnosed borderline diabetic (Fasting blood glucose > 110 mg/dl)
- Body Mass Index (BMI) ≥ 28
- Resting HR < 50 or > 100 bpm
- Orthopedic problem that would inhibit submaximal effort on the cycle ergometer

The three groups were matched for age, body weight, height, BMI, and body surface area (BSA). Descriptive statistics of the three groups are found in Table 3.1. Signed informed consents were obtained from all participants. A copy of the informed consent was provided to each participant. This study was approved by the Institutional Review Board of Louisiana State University and the Research and Development Council of Woman's Hospital.

Table 3.1. Descriptive Statistics of the Three Groups of Subjects.

Variable	Group M (n=11)	Group F (n=14)	Group G (n=7)
Age (yr)	31.5 ± 5.66 (21-39)	31.3 ± 8.5 (21-42)	27.8 ± 3.7 (24-34)
Body Weight (kg)	60.2 ± 5.4 (51.4-69.8)	60.4 ± 7.6 (49-76.1)	64.6 ± 10.0 (54.5-77.2)
Height (cm)	163.0 ± 5.2 (152-171)	165 ± 5.9 (156-180)	166 ± 7.1 (152-173)
BMI (kg/m^2)	22.6 ± 2.3 (19.6-27.4)	22.1 ± 2.0 (18.9-27.0)	23.3 ± 2.5 (19.9-25.9)
BSA (m^2)	1.8 ± 0.11 (1.70-2.09)	1.8 ± 0.15 (1.71-2.21)	1.9 ± 0.20 (1.77-2.23)

Values are means ± SEM (range);
No statistically significant differences were found among groups
BMI = Body Mass Index
BSA = Body Surface Area

Experimental Procedures

Volunteers were recruited by word of mouth, flier advertisement, and physician referral. Preliminary screening by telephone confirmed inclusion and exclusion criteria. If the subject was deemed appropriate for the study, and was still interested in participating, a meeting was scheduled for the purpose of explaining the study's requirements in detail, signing the informed consents, and completing the physical fitness testing to be used in the Physical Fitness Profile (PFP). The PFP was provided to each subject as an incentive to participate in the study and was not included in the data collection. The sequence of this preliminary testing was as follows:

1) Determination of body weight and height, using upright scales (Health-O-meter, Baumann), and calculation of BMI (kg/m^2);

2) Determination of body fat from the sum of three skinfold measurements (suprailium, thigh, and triceps) using Lange Skinfold Calipers; standard values used to determine fitness level were those of The Institute for Aerobics Research in ACSM's *Guidelines for Exercise Testing and Prescription*, 5th Edition, 1995, p. 112;

2) Muscular strength determined by 1 repetition max (1RM) tests on the Nautilus leg press machine and the Body Masters bench press; standard values used to determine fitness levels were those of The Institute for Aerobics Research in ACSM's *Resource Manual for Guidelines for Exercise Testing and Prescription*, 2nd Edition,1993, p.241;

3) Flexibility determined by sit-and-reach test; standard values used to determine fitness level were those of The Institute for Aerobics Research in ACSM's *Guidelines for Exercise Testing and Prescription*, 5th Edition, 1995, p. 128;

4) Lung function determined by spirometry: FEV_1/FVC (Pneumoscan S-300 Spirometer);

5) Resting 12-lead electrocardiogram, reviewed by a board-certified cardiologist;

6) Dual Energy X-ray Absorptiometry (DEXA) for determination of bone density of the lumbar spine and proximal femur using QDR-1000 (Hologic, Waltham, MA);

7) Nutritional assessments were performed using Nutritionist IV computer software, Version 4, First DataBank;

Subjects were then scheduled to perform the submaximal cycle ergometer test to be performed either during their menses (day 1-4) or during the follicular phase (day 8-12) whichever came first and was convenient. They were advised

- to abstain from food and drink (other than water) after midnight the evening before testing
- to avoid moderate or vigorous physical activity within 12 hours of the test
- to empty bladder prior to testing
- to abstain from alcohol consumption within 48 hours of the test

- to ingest no over-the-counter drug, *eg.*, aspirin, ibuprofen, or decongestant, etc., within 24 hours of the test.

Equipment used in the cycle ergometer test were the QMC™ metabolic cart (Quinton Instrument Co., Bothell, WA.), Quinton electronically braked cycle ergometer analog, and Quinton electrocardiogram heart rate analog.

Before each test, the QMC™ metabolic cart's power and analyzer switches were turned on and allowed to warm up for thirty minutes before calibrating the pneumotach and O_2 and CO_2 gas analyzers with standard grade gas mixtures, according to manufacturer's instructions. The room environment was kept constant during the study period: 72 °F (22.2°C), ~50% humidity.

Upon arrival at the testing laboratory at 7:00 a.m. the subject was prepped for the placement of 5 electrodes (LL, LA, RL, RA, and V_5) for a continuous monitor of electrocardiogram and heart rate analog input. Resting blood pressure was measured by auscultation, using a mercury sphygmomanometer on a rolling base. The subject was given instructions on how to breathe through the mouthpiece. The headgear and nose clip were placed on the subject, and she was asked to sit quietly in a chair with her legs elevated in the semi-Fowler position. After 15 minutes of resting data collection, the headgear, mouthpiece, and nose clip were removed, and a catheter was inserted into an antecubital vein in the arm with a stopcock in the line and a 50 cc bag of normal saline set at a keep-open rate. The first blood sample (baseline) was collected at this time.

The subject was then asked to sit on the cycle ergometer in order to set seat height (5-20 degrees flexion). The breathing apparatus was re-attached, and

the subject was asked to begin pedaling at 50 RPM's at zero resistance (zero watts). After two minute stages, the workload was increased by 10 watt increments, and this was continued every 2 minutes until 60% of the age-predicted maximal heart rate (220 - age x 60%) was achieved (60%HR). Blood pressure and rating of perceived exertion (RPE using Borg scale 6-20) were documented at each stage. At 10 minutes the second blood sample was drawn. Upon reaching 60%HR, the subject was asked to continue pedaling at 50 RPM's for the remainder of the 20 minute test. Workload was continually assessed and changed to maintain steady state at the 60% goal. The third blood sample was drawn at the conclusion of the work test (20 minutes). Indications for stopping the tests were the following (*ACSM Guidelines*, 1995):

1. Onset of angina or angina-like symptoms
2. Significant drop (20 mm Hg) in systolic blood pressure or a failure of the systolic blood pressure to rise with an increase in exercise intensity
3. Excessive rise in blood pressure: systolic pressure >260 mm Hg or diastolic pressure > 115 mm Hg
4. Signs of poor perfusion: lightheadedness, confusion, ataxia, pallor, cyanosis, nausea, or cold and clammy skin
5. Failure of heart rate to increase with increased exercise intensity
6. Noticeable change in heart rhythm
7. Subject requests to stop
8. Physical or verbal manifestations of severe fatigue
9. Failure of the testing equipment

The mouthpiece, noseclip, and supporting headgear were removed immediately after testing, and the subject was asked to cool down gradually, still

pedaling at a slow rate, with no workload. The catheter and IV were then removed and the subject was offered oral fluids.

Indirect Calorimetry

As opposed to direct calorimetry which is the measurement of heat production as a result of energy expenditure, indirect calorimetry measures the rate of oxygen uptake or oxygen consumption (VO_2). Taken alone, VO_2 provides a measure of the amount of energy expended (ACSM, 1995). When combined with concurrent measurements of carbon dioxide production (VCO_2), oxygen consumption provides information about the type of fuel being used, *e.g.*, fat or carbohydrate.

In this study, respiratory gases were collected and analyzed using open spirometry on a breath-by-breath basis, and averaged over 30 second periods, using the QMC™ Software, version 2.10. Volume of air breathed, minute ventilation (VE), was reported in liters per minute (L/min), volume of oxygen consumed was reported in absolute terms (L/min) and relative terms (ml/kg/min). The volume of carbon dioxide produced was reported in liters per minute.

The R value was calculated and reported by the QMC using the ratio of VCO_2/VO_2. Table 3.2 is the culmination of detailed research performed by early investigators in the field of nutrition and metabolism. It depicts the range of R values from 0.707 representing no oxygen being consumed by carbohydrate and all of the oxygen being consumed by fat, utilizing 4.686 calories per liter of oxygen, to a value of 1.00, representing 100% oxygen being consumed by carbohydrate, no oxygen being consumed by fat, and utilizing 5.047 calories per liter of oxygen.

Table 3.2. Analysis of the Oxidation of Mixtures of Carbohydrate and Fat

R.Q.	Percentage of total oxygen consumed by:		Percentage of total heat (Calories) produced by:		Calories per liter O_2	
	Carbohydrate	Fat	Carbohydrate	Fat	Number	Logarithm
0.707	0	100.0	0	100.0	4.686	0.67080
0.71	1.02	99.0	1.10	98.9	4.690	0.67114
0.72	4.44	95.6	4.76	95.2	4.702	0.67228
0.73	7.85	92.2	8.40	91.6	4.714	0.67342
0.74	11.3	88.7	12.0	88.0	4.727	0.67456
0.75	14.7	85.3	15.6	84.4	4.739	0.67569
0.76	18.1	81.9	19.2	80.8	4.751	0.67682
0.77	21.5	78.5	22.8	77.2	4.764	0.67794
0.78	24.9	75.1	26.3	73.7	4.776	0.67906
0.79	28.3	71.7	29.9	70.1	4.788	0.68018
0.80	31.7	68.3	33.4	66.6	4.801	0.68129
0.81	35.2	64.8	36.9	63.1	4.813	0.68241
0.82	38.6	61.4	40.3	59.7	4.825	0.68352
0.83	42.0	58.0	43.8	56.2	4.838	0.68463
0.84	45.4	54.6	47.2	52.8	4.850	0.68573
0.85	48.8	51.2	50.7	49.3	4.862	0.68683
0.86	52.2	47.8	54.1	45.9	4.875	0.68793
0.87	55.6	44.4	57.5	42.5	4.887	0.68903
0.88	59.0	41.0	60.8	39.2	4.899	0.69012
0.89	62.5	37.5	64.2	35.8	4.911	0.69121
0.90	65.9	34.1	67.5	32.5	4.924	0.69230
0.91	69.3	30.7	70.8	29.2	4.936	0.69339
0.92	72.7	27.3	74.1	25.9	4.948	0.69447
0.93	76.1	23.9	77.4	22.6	4.961	0.69555
0.94	79.5	20.5	80.7	19.3	4.973	0.69663
0.95	82.9	17.1	84.0	16.0	4.985	0.69770
0.96	86.3	13.7	87.2	12.8	4.998	0.69877
0.97	89.8	10.2	90.4	9.58	5.010	0.69984
0.98	93.2	6.83	93.6	6.37	5.022	0.70091
0.99	96.6	3.41	96.8	3.18	5.035	0.70197
1.00	100.0	0	100.0	0	5.047	0.70303

Source: Lusk, G. *The Elements of the Science of Nutrition*, 4th Ed., 1976 , p.65

See Figure 3.1. for formulas used to obtain these values. The percentage of total heat produced (kilocalories) by carbohydrate (%CHO) and the number of kilocalories expended per liter of oxygen (kcal/LO_2) were computed by SAS using these formulas. Using simple linear regression of percent carbohydrate utilization at workloads of 10, 20, 30, and 40 watts, the crossover points were established for each subject and then averaged for each group.

Analysis of Blood Samples

Three sets of blood samples of approximately 20 ml each, were withdrawn from the stopcock system and placed in a 20cc glass test tube. A microcapillary tube was immediately filled and set aside for analysis after the test. Approximately two ml of whole blood was also placed into a green-topped vacutainer containing sodium heparin to be used to analyze for plasma ammonia, lactate and glucose, and 0.5 ml was placed in two ml chilled perchloric acid for possible use in later serum analyses. The remaining blood was allowed to coagulate in the large collection tube. All tubes were placed on ice until the completion of the test. The tubes were then centrifuged at 10,000 RPM for 10 minutes, and alliquots of the plasma and serum were placed into labeled glass test tubes. Samples were placed in a -80 degrees centigrade freezer for future analysis. A sample was sent to the lab for analysis of lipids on the same day as testing. Each type of assay was performed on all samples on the same day, and with kits using the same lot numbers, to avoid interassay variations. Also, all samples were thawed only once for each assay procedure. Hematocrit was measured using microcapillary tubes centrifuged at 11,000 for 5 minutes within 15 minutes after testing.

Percentage (%) of total oxygen consumed by carbohydrate = $100\ [(R - 0.707) / 0.293]$

Percentage (%) of total oxygen consumed by fat = $100\ [(1.00 - R) / 0.293]$

Percentage (%) of total heat produced (Calories expended) by carbohydrate = $\{[504.7\ (R - 0.707)] / [5.047\ (R - 0.707) + 4.686\ (1.00 - R)]\}$

Percentage (%) of total heat produced (Calories expended) by fat = $\{[468.6\ (1.00 - R)] / [5.047\ (R - 0.707) + 4.686\ (1.00 - R)]\}$

Calories (C) per liter oxygen = $4.686 + [(R - 0.707) / 0.293] * 0.361$

The logarithm of Calories = log of the number of Calories calculated above

Figure 3.1. **Formulas used to derive mixtures of carbohydrate and fat utilization. (Refer to Table 3.1)**

Levels of serum total cholesterol (TC), HDL cholesterol, and triglycerides (TRIG) were measured directly by colorimetry using the automated Vitros 700 Analyzer and Vitros CHOL Slides, Vitros HDL Cholesterol Kit, and TRIG Slides, respectively (Johnson & Johnson Clinical Diagnostics, Inc., Rochester, NY). The method for measuring TC is based on an enzymatic method similar to that proposed by Allain and associates (1974). The method for measuring HDL is described previously (Warnick, *et al.*, 1983). The method for measuring TRIG is based on an enzymatic method as described by Spayd and associates (1978). Within lab precision (Coefficient of Variation = CV%) was determined to be 1.5-4.1 for TC, 1.5-4.4 for HDL, and 1.4-1.9 for TRIG. Sensitivity (the lower limit of the reportable, dynamic range) was 50 mg/dl for TC, 0.1 mg/dl for HDL, and 10.0 mg/dl for TRIG. LDL cholesterol levels were calculated using the formula: LDL = Total Cholesterol - [HDL + (Triglyceride/5)] according to Tietz (1990).

Estradiol levels in the blood were measured using an automated immunoassay analyzer (IM®x Abbott Diagnostics, North Chicago, IL) and Microparticle Enzyme Immunoassay (MEIA) technology. The estradiol kit was obtained from Abbott Laboratories (Diagnostics Division, Abbott Park, IL 60064). The sensitivity of the Imx Estradiol assay was previously calculated to be 15 pg/ml (the lowest measurable concentration of estradiol that can be distinguished from zero). The coefficients of variation (%CV's) for within assay, between assay and total assay precision were determined to be 10.4, 16.0, and 19.1 respectively, for mean value of 67; %CV's = 6.3, 6.7, and 9.2 for mean value of 140; %CV's = 3.8,

4.3, and 5.7 for mean value of 524; and %CV's = 4.1, 4.5, and 6.1 for mean value of 1166 (Krouwer & Rabinowitz, 1984).

Plasma glucose (GLU), ammonia (AMON or NH_3), and lactate (LAC) were determined by colorimetry, using the automated Vitros 700 Analyzer and Vitros GLU Slides, AMON Slides, and LAC Slides, respectively (Johnson & Johnson Clinical Diagnostics, Inc., Rochester, NY). The dye system used in measuring GLU is related to that reported by Trinder (1969), and the chemistry of the glucosed slides used has previously been described (Curme, *et al.*, 1978). The reaction sequence used in the measurement of AMON is: NH_3 + bromophenol blue----->blue dye (spectrophotometrically measured at 600 nm). The reaction sequence used in the measurement of LAC is: L-(+)-lactic acid + O_2------*lactate oxidase*------>pyruvate + H_2O_2---------->2 H_2O_2 + 4-aminoantipyrine + 1,7-dihydroxynaphthalene----*peroxidase*--->red dye (spectrophotometrically measured at 540 nm). Within lab precision was previously determined to be 1.0-1.7 CV% for GLU, 1.6-10.8 CV% for AMON, and 1.3-1.8 CV% for LAC, respectively. Sensitivity was previously determined to be 20.0 mg/dl for GLU, 1.0 µmol/L for AMON, and 4.51 mg/dl for LAC.

Serum cortisol was measured by Fluorescence Polarization Immunoassay (FPIA) using the automated TDx™ analyzer and the TDx/TDxFLx cortisol assay reagent system (Abbott Laboratories Diagnostics Division, Abbott Park, IL). Sensitivity was previously determined to be 0.64 µg/dl (Abbott Laboratories).

Serum insulin was determined by MEIA using the IM®x automated immunoassay analyzer and IM®x insulin reagents (IM®x Abbott Diagnostics, North

Chicago, IL). The %CV's for within assay, between assay, and total assay precision were all less than 6.0 (Krouwer & Rabinowitz , 1984).

Experimental Statistics and Design

Resting and exercise data were entered as separate tests for each subject. Data were depicted descriptively and outliers (>2 standard deviations from group mean) eliminated before inferential statistics were applied. Microsoft EXCEL for windows, v7.0, was used to convert the QMC™ ASCII files to SAS® files (SAS System for Windows™ v 6.12; Cary, NC); GB Stat for Windows, v 5.0 (Dynamic Microsystems, Inc., Friedman, 1993), and SAS® were used for descriptive analyses and statistical procedures. Significance was set *a priori* at $p<0.10$. Tukey's *post hoc* tests were performed when significance was found. Unless otherwise stated, results are reported as means ± SD. A *post hoc* power analysis was performed on the criterion R values which resulted in $\phi = 0.90$.

Mean values of the dependent variables of the three groups, M, F, and G (eumenorrheic @ menses, eumenorrheic @ follicular, and hypoestrogenemic due to GnRHaa therapy, respectively) were compared during rest, during each stage of exercise (0, 10, 20, 30, 40 Watts) and during the last 5 minutes of exercise using a one way analysis of variance (ANOVA). Multiple regression analysis was also used in an effort to fully describe the proportion of variability in the R value derived from estrogen level, BMI, and age.

CHAPTER 4. RESULTS

Results of data collection are divided into 5 separate studies: (1) at rest; (2) at absolute workloads, after 2 minutes of freewheeling warmup (denoted as 0 watts, herein) of 10, 20, 30, and 40 watts; (3) at steady-state of 60% age-predicted maximum heart rate; (4) blood analyses; and (5) crossover point analysis . Data in the rest and exercise studies are further delineated by cardiorespiratory and metabolic parameters. Data are presented in tabular form as group means ± standard deviation, as well as in graphic form. Significance is denoted with a superscript and foot-noted. Refer to the Appendix for individual results.

Resting Study

The resting portion of the study comprised of 15 minutes of data collection, of which the first two minutes were eliminated due to the number of outliers. There were no significant differences found among groups for the cardiorespiratory variables of VE, absolute VO_2 and VCO_2. However, group G demonstrated the smallest relative VO_2, differing significantly from group M ($p=0.0395$). Group G also demonstrated a lower resting heart rate (HR) than both group F ($p=0.0415$) and group M ($p=0.0549$), as well as a lower percentage age-predicted HR (%HR) than group F ($p=0.0344$) and group M ($p=0.0332$). (Refer to Table 4.1.)

There were no significant differences among group means for the metabolic variables (Table 4.2): R, %CHO/FAT utilized, kilocalories expended per liter of oxygen ($kcal/O_2$), kilocalories expended per minute (kcal/min), and resting energy expenditure per day (REE) in kilocalories.

Table 4.1. Group Means of Cardiorespiratory Variables at Rest.

Variable	Group M	Group F	Group G
VO_2stpd (L/min)	0.15 ± 0.03	0.14 ± 0.03	0.12±0.03
VO_2 (ml/kg/min)	2.54±0.50	2.11±0.40	1.79±0.70 [a]
VCO_2stpd (L/min)	0.14±0.02	0.13±0.04	0.10±0.02
VEbtps (L/min)	4.10±1.70	3.33±1.36	3.07±1.50
HR (BPM)	74.0± 8.2	76.0± 10.3	62.6± 8.4 [b]
%HRmax	39.1±4.2	39.8±5.5	32.5±4.2 [c]

Values are mean ± standard deviation.

[a] Different from Group M (p=0.0395).
[b] Different from Group F (p= 0.0415) and Group M (p=0.0549).
[c] Different from Group F (p=0.0344) and Group M (p=0.0332).

Table 4.2. Group Means of Metabolic Variables at Rest.

Variable	Group M	Group F	Group G
R	0.883 ± 0.06	0.891 ± 0.074	0.89 ± 0.08
%CHO	61.8 ± 21.03	64.5 ± 24.76	64.9 ± 25.00
kcal/LO_2	4.90 ± 0.08	4.91 ± 0.09	4.91 ± 0.09
kcal/min	0.78 ± 0.17	0.72 ±0.19	0.61 ±0.17
REE(kcal/day)	1122.00 ±247.68	1039.81±270.77	870.28±240.56

Values are mean ± standard deviation.
No Significant Differences among means.

Graded Workload Study

Variable responses were analyzed at the second minute of each stage after 2 minutes of freewheeling at 0 watts, *i.e.*, second minute at 10, 20, 30 and 40 watts. There were no differences among groups for most of the cardiorespiratory variables. The two exceptions were (1) group G had a significantly lower VE at 40 watts than group M ($p=0.0684$), and (2) during the 10 watt workload, %HR for group G was lower and close to significance (group F: $p=0.0525$ and group M: $p=0.0933$). There were no significant differences among group means for the metabolic data. Refer to Tables 4.3 and 4.4.

Steady-state Study

After 10 minutes of graded exercise (increasing workloads), the next 5 minutes were allowed to achieve steady state at 60% of age-predicted maximum HR, and then the variable response was analyzed for the last 5 minutes of exercise. No significant differences were found among group means for either the cardiorespiratory or metabolic variables measured. Refer to Tables 4.5 and 4.6.

Total Work

Group G differed also in the amount of work performed during exercise at steady state. The group had a significantly higher power output in watts, differing from group F ($p=0.0006$) and group M ($p=0.0026$), and in kilopond meters (KPM's), differing from group F ($p=0.0020$) and group M ($p=0.0029$). Refer to Table 4.7.

Table 4.3. Summary of Group Means of Cardiorespiratory Variables at 0, 10, 20, 30, and 40 Watts.

VARIABLE	Watts	GROUP M	GROUP F	GROUP G
VO_2stpd (L/min)	0	0.22±0.05	0.25±0.06	0.24±0.04
	10	0.25±0.06	0.26±0.06	0.26±0.06
	20	0.30±0.05	0.28±0.07	0.31±0.07
	30	0.38±0.05	0.36±0.08	0.35±0.10
	40	0.51±0.07	0.50±0.08	0.46 0.13±
VO_2 (ml/kg)	0	3.7±0.8	4.1±1.3	3.8±0.8
	10	4.2±1.0	7.7±17.6	3.9±1.4
	20	4.9±0.9	4.9±1.3	4.8±1.1
	30	6.4±1.1	6.1±1.3	5.5±1.5
	40	8.6±1.6	8.5±1.4	7.3±1.9
VCO_2stpd (L/min)	0	0.17±0.04	0.19±0.05	0.19±0.03
	10	0.20±0.05	0.20±0.05	0.20±0.05
	20	0.24±0.04	0.23±0.06	0.25±0.06
	30	0.33±0.05	0.31±0.07	0.29±0.09
	40	0.47±0.08	0.45±0.08	0.40±0.12
VEbtps (L/min)	0	5.58±1.8	4.71±2.1	5.13±2.7
	10	6.89±1.8	5.91±2.2	5.37±2.8
	20	8.59±1.8	7.09±2.6	7.14±2.9
	30	11.50±2.2	9.91±2.4	9.00±3.2
	40	15.56±3.2	13.60±2.9	12.12±3.7 [a]
HR (BPM)	0	89.3±8.0	90.2±8.5	82.9±8.4
	10	94.6±9.3	94.6±9.3	85.7±8.0
	20	98.2±8.0	98.5±10.3	91.9±8.5
	30	106.8±8.2	106.5±9.7	98.6±10.1
	40	117.8±11.3	116.5±11.2	107.6±9.3
%HRMAX	0	47.2±4.2	47.6±4.1	43.2±4.2
	10	49.6±4.2	49.8±4.4	44.6±4.0 [b]
	20	51.8±4.0	51.6±5.0	47.8±4.1
	30	56.2±4.2	56.1±5.0	51.3±5.1
	40	62.0±6.0	61.3±5.8	56.2±4.8

Values are mean ± standard deviation.

[a] Different from Group M (p=0.0684).

[b] Different from Group F (p=0.0525) and Group M (p=0.0933).

Table 4.4. Summary of Group Means of Metabolic Variables at 0, 10, 20, 30, and 40 Watts.

VARIABLE	Watts	GROUP M	GROUP F	GROUP G
R	0	0.76±0.06	0.75 ±0.06	0.78 ±0.05
	10	0.81 ±0.04	0.79 ±0.05	0.79 ±0.04
	20	0.83 ±0.04	0.80 ±0.05	0.80 ±0.03
	30	0.87 ±0.06	0.84 ±0.08	0.83 ±0.03
	40	0.92 ±0.08	0.90 ±0.07	0.92 ±0.03
%CHO	0	20.97±22.3	16.31±19.2	28.30±18.1
	10	36.71±14.6	26.42±21.0	28.57±17.1
	20	44.34±14.3	31.82±20.6	35.74±13.6
	30	58.14±21.1	48.21±21.7	44.68±12.1
	40	72.56±24.4	63.71±24.3	59.55±10.23
kcal/LO_2	0	4.75±0.07	4.74±0.06	4.78±0.06
	10	4.81±0.05	4.77±0.07	4.78±0.06
	20	4.84±0.05	4.79±0.07	4.80±0.04
	30	4.89±0.07	4.85±0.07	4.84±0.04
	40	4.94±0.09	4.91±0.08	4.89±0.03
kcal/min	0	1.05±0.22	1.15±0.37	1.07±0.26
	10	1.15±0.36	1.27±0.46	1.30±0.48
	20	1.45±0.51	1.45±0.51	1.50±0.51
	30	1.90±0.30	1.81±0.58	1.57±0.51
	40	2.50±0.51	2.54±0.50	2.28±0.72

Values are mean ± standard deviation.
No Significant Differences among group means.

Table 4.5. Group Means of Cardiorespiratory Variables at 60 % Age-predicted Maximum Heart Rate (Last 5 Minutes of Exercise)

VARIABLE	GROUP M	GROUP F	GROUP G
VO_2 stpd (L/min)	0.46±0.19	0.43±0.18	0.51±0.15
VO_2 (ml/kg)	7.64±3.11	7.52±3.32	8.10±2.27
VCO_2 (L/min)	0.41±0.17	0.39±0.19	0.46±0.14
VEbtps (L/min)	13.82±5.30	12.34±4.59	14.37±3.82
HR (BPM)	116.37±4.90	118.67±5.57	118.48±3.43
%HRMAX	61.41±1.66	61.9±1.66	61.78±1.32

Values are mean ± standard deviation.
No Significant Differences among group means.

Table 4.6. Group Means of Metabolic Variables at 60% Age-predicted Maximum Heart Rate (Last 5 minutes of exercise)

VARIABLE	GROUP M	GROUP F	GROUP G
R	0.891 ± 0.04	0.888 ± 0.06	0.908 ± 0.03
%CHO	64.39 ± 15.79	63.2 ± 16.82	70.11 ± 12.12
kcal/LO_2	4.91 ± 0.05	4.91 ± 0.06	4.93 ± 0.04
kcal/min	2.28 ±1.05	2.11± 0.94	2.55 ± 0.76

Values are mean ± standard deviation.
No Significant Differences among group means.

Blood Analysis

Estrogen status was confirmed, and as expected, groups M ($p<0.05$) and G ($p<0.01$) were significantly lower than group F. Refer to Table 4.8 for results of blood analysis data and Figure 4.1 for a graphic representation of estrogen status at rest. There were no significant differences among group means for cortisol, glucose or hematocrit. Some differences were found within groups, however, in the remaining metabolic substrates of lactate and ammonia. Resting values were lower than the exercise values for ammonia in group M ($p<0.05$), and for lactate in group F ($p<0.05$) and group M ($p<0.01$).

Crossover Points

The crossover point was estimated to be 30.8 (±25.44) watts for group M, 34.79 (±26.53) watts for group F, and 32.85 (±7.33) watts for group G. The differences were not statistically significant. Refer to Table 4.9.

Diet Analysis

An analysis of the 24 hour food intake prior to testing revealed no significant differences among groups for total kilocalories consumed, percent of kilocalories in the macronutrients of fat (%Fat), protein (%Pro), or carbohydrates (%CHO). Refer to Table C.1 in the Appendix for individual values and means per group.

Multiple Regression Analysis

Multiple regression was performed and revealed no significant contribution of estrogen status, BMI, or age to the R value.

Table 4.7. Group Means of Work Performed During Submaximal Ergometer Test at settings of 0, 10, 20, 30, 40 Watts and @ 60% Age-Predicted Maximum Heart Rate in Watts and KPMs.

Work	Settings	Group M	Group F	Group G
Watts	0	0.50±0.51	0.77±0.50	0.35±0.49
	10	10.40±0.59	10.40±0.50	10.40±0.50
	20	20.20±0.41	20.36±0.56	20.21±0.42
	30	30.50±0.51	31.25±2.56	30.57±0.51
	40	40.25±0.44	39.96±3.99	40.00±0.00
	60%	31.24±12.87	30.83±14.27	37.85±8.36
KPMs	0	3.2±0.69	3.47±1.70	2.64±0.84
	10	63.75±2.82	63.69±2.89	63.69±2.89
	20	124.65±2.56	124.09±2.94	123.85±2.56
	30	185.6±1.66	191.52±16.71	186.07±1.77
	40	246.55±2.35	245.08±25.90	245.78±1.36
	60%	190.98±79.31	190.40±94.47	232.34±50.54

Values are mean ± standard deviation.
No Significant Differences among means.

Table 4.8. Group Means of Blood Analyses.

	Time of Draw	Group M	Group F	Group G
Estrogen (pg/ml)	@Rest (0)	56.43± 27.4 [a]	217.31±236.25	7.31±6.98 [b]
Total Cholesterol (mg/dl)	@Rest (0)	158.5±15.1	169.4 ±27.6	160.6±33.6
HDL (mg/dl)	@Rest (0)	48.3 ±13.0	56.5 ±7.6	53.5 ±8.0
LDL (mg/dl)	@Rest (0)	91.8 ±12.7	99.2 ±21.9	93.9 ±29.0
Triglycerides (mg/dl)	@Rest (0)	92.0 ±71.2	68.5 ±22.9	66.0 ±28.1
Glucose (mg/dl)	0	86.8 ±4.4	82.4 ±12.5	90.1 ±10.4
	10	86.0 ±5.3	85.0 ±8.1	91.8 ±9.1
	20	83.8 ±7.3	82.3 ±14.0	90.5±9.5
Ammonia (µmol/L)	0	18.6 ±6.2 [c]	25.2 ±11.9	24.6 ±7.3
	10	22.0 ±5.4	28.5 ±16.6	32.6 ±10.4
	20	25.5 ±5.8	36.3 ±31.1	34.5 ±10.5
Lactate (mmol/L)	0	1.1 ±0.3	1.1 ±0.3 [d]	1.0 ±0.2*
	10	1.9 ±1.0	1.9 ±0.8	1.9 ±0.2
	20	1.8 ±1.2	2.2 ±1.1	1.9 ±0.2
Cortisol (µg/dl)	0	14.13 ±4.76	16.82 ±6.88	14.34 ±2.88
	10	13.58 ±4.85	17.09 ±5.99	13.78 ±3.91
	20	12.09 ±4.66	16.42 ±4.71	13.40 ±3.81

Values are mean ± standard deviation.

[a] Different from Group F ($p<0.05$).
[b] Different from Group F ($p<0.01$).
[c] Different from Within group @20 ($p<0.05$).
[d] Different from Within group @10 ($p<0.01$) and @20 ($p<0.05$).
* Different from Within group @10 and @20 ($p<0.01$).

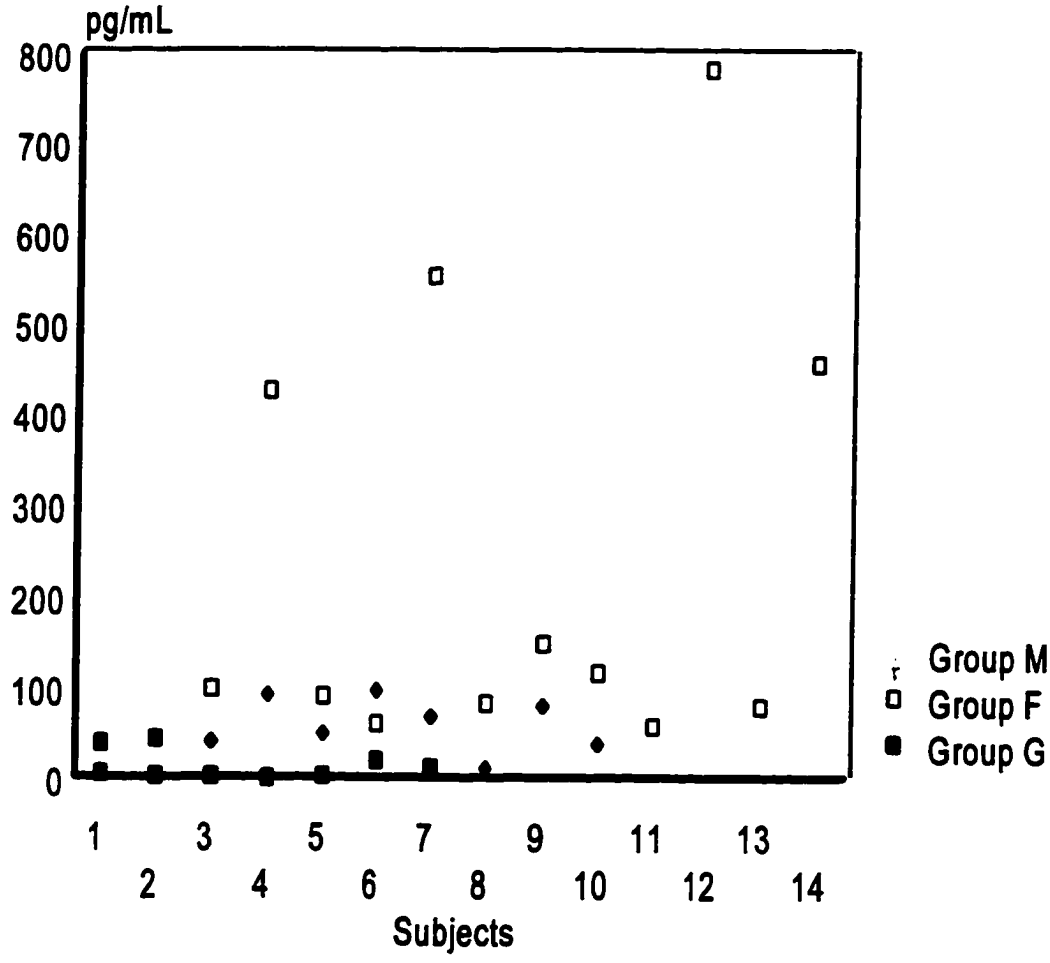

Figure 4.1. Serum Estrogen Levels of All Subjects at Rest (pg/ml)

Table 4.9. Calculated Crossover Points of Subjects (Watts)

Subject	Group M	Group F	Group G
1	6.47	48.69	27.47
2	18.98	42.63	40.31
3	18.74	26.75	43.57
4	29.19	25.56	27.91
5	5.71	31.95	24.91
6	42.00	53.54	28.67
7	42.94	38.65	37.14
8	22.55	50.15	
9	93.75	101.28	
10	27.65	23.10	
11		12.99	
12		39.16	
13		-11.44	
14		4.07	
Group Mean±SD	30.80±25.44	34.79±26.53	32.85±7.33

No Significant Differences among group means.

CHAPTER 5. DISCUSSION

The main purpose of this study was to examine the influence that estrogen status, *i.e.*, chronically low, acutely low and acutely high concentrations as found in postmenopausal, menses and follicular phase, has on fuel substrate utilization at rest, and at specific absolute and relative submaximal workloads.

During the resting metabolic and cardiorespiratory studies, the three groups demonstrated little variation among their VO_2 (l/min) and R means. Since the other resting metabolic variables, %CHO, kcal/LO_2, kcal/min and REE were all based on the R value, significant differences would not be expected. While no differences were found in absolute oxygen consumption, there was a significant difference found in the relative oxygen consumption: Group G was significantly lower than group M, but not group F. These results are directly influenced by the weight of the subjects, *i.e.*, given the same absolute oxygen consumption, the heavier individuals will have a lower relative oxygen consumption. Even though there were no significant differences found among the average body weights of the groups, group G was heaviest ($p=0.4263$). The lowest absolute oxygen consumption coupled with the highest body weight probably led to the significant difference in relative oxygen consumption.

The R value of group G was one point (0.01) higher than that of the other two groups, but was nevertheless insignificant. Kanaley and associates (1992) corroborates these findings. They tested two groups of women athletes, one eumenorrheic group ($n=7$) which was tested three times--at the early follicular phase, late follicular phase, and the mid-luteal phase of their menstrual cycles,

and compared the results to an amenorrheic group (n=6). Even though their findings were not significant, higher R values were found for the amenorrheic and early follicular subjects compared to the late follicular (and luteal) subjects at rest. They nevertheless concluded that resting carbohydrate and fat utilization were not affected by phase-related differences in estrogen.

With respect to the other resting variables, heart rate and percentage of age-predicted maximum heart rate were significantly different among groups. Group G responded with lower values when compared with the other two groups. There are conflicting results from earlier studies regarding heart rate response during different phases of the menstrual cycle. An early study reported that heart rate was lower during the menses than during other phases (Cullis, *et al*, 1922). Phillips (1966), however, reported that menstrual cycle phases did not have an effect on pulse rate. Differences in resting heart rate might be explained by higher fitness levels, but this factor probably is not the reason for the differences seen in this study since a screening process was used to ensure that the participants had similar activity levels. Moreover, it seems likely that if they were more fit, they would have responded with lower heart rates at any workload, but their response to increased workloads in the graded portion of the test were similar to those in the other groups. Another possible reason that differences were seen in resting HR is that group G was slightly larger in body size, and hence decreased HR could be due to group G having a slightly larger stroke volume. It is also possible that the resting portion of the study was not a long enough period of time to elicit true resting heart rate values. It is interesting to note that no studies have investigated

the effects of GnRHaa on resting HR. It has been reported by TAP Pharmaceuticals, Inc. (1997) that side effects of GnRHaa therapy include a small percentage of patients presenting with tachycardia and palpitations and no reports of depressed heart rate *per se*. Therefore, the possibility of GnRHaa being a cardiodepressive cannot be discounted, but it is unlikely.

It is important to note that even though there was no statistical significance between metabolic variables, a small difference could have considerable consequences over a period of time. The long term effects of the small metabolic differences, as shown in the results of this study, can be easily calculated. For instance, if a sedentary premenopausal female has been consuming a 1500 kcal diet and maintaining her weight, it is assumed that her energy expenditure is approximately equal to 1500 kcal. Her basal or resting energy expenditure would be 1039.81 kcal (based on group F resting values), with the remaining 460.19 kcal attributed to light daily activity. Now assuming she has become postmenopausal and that her activity (460.19 kcal) and her energy consumption (1500 kcal) have not changed, her resting energy expenditure would drop to approximately 870.28 kcal (group G value at rest) . Now, when her resting energy expenditure is added to her normal unchanged activity of 460.19 kcal, the total caloric expenditure is 1330.47 kcal, 169.53 kcal less than the premenopausal daily expenditure. Hence, over a 30 day period, she would consume approximately 5086 kcal more than her maintenance weight would require. Since one pound of fat is equal to 3500 kcal, it is conceivable that once a woman reached menopause, she could gain approximately 1.45 pounds of fat weight per month totaling about 17 pounds in

one year. Of course, a woman does not normally jump from premenopause to postmenopause overnight. It is a transition over a period of months and even years. So any weight gain due to a decreased resting expenditure would be a gradual phenomenon, and noticeable only after a period of months. Hence, an acute study may not be the most appropriate model to investigate the influences of estrogen. A prolonged longitudinal study would need to be employed to document the alteration in body composition suggested above.

During the graded workload study, all three groups again responded similarly in both the metabolic study and the cardiorespiratory study. There were two anomalies, with group G responding with a lower minute ventilation than group M only at 40 watts, and the same group responding with a lower %HR than group M and group F only at 10 watts. According to this study's protocol, workload was begun at zero watts for two minutes as a warmup and then workload was increased by 10 watts every 2 minutes (until ~50 watts). The rate with which work is incremented can have a profound influence on oxygen uptake and, moreover, individuals vary considerably in their ability to adapt to a given workload (Gullestad, *et al.*, 1997). Statistically significant differences have been found in R between 1 minute and 3 minute stages on a cycle ergometer when increases are between 40 and 70 watts (Gullestad, *et al.*, 1997). It is desirable to reach steady state at each stage, that is, to allow time for respiration and circulation to adjust to the new intensity. At steady state, oxygen uptake corresponds to the demands of the tissues (oxygen uptake = oxygen requirement), and carbon dioxide production is stabilized. Otherwise, true metabolic status cannot be measured. According

to Åstrand & Rodahl (1986), a stage of at least 5 minutes is required to obtain steady state conditions. In this study, even though the incremental workloads were very small compared to other submaximal cycle ergometer protocols, and steady state was expected to occur, it is likely that the two minute stages were not long enough to elicit steady state in this untrained population and thus hindered the detection of metabolic differences.

No significant differences were found among group means at steady state exercise and therefore the results of this study do not support the hypothesis that there would be differences. There are several possible reasons for the lack of significance. The extremely low oxygen consumption values, even during exercise, were surprising. However, they were substantiated using the metabolic formula for oxygen consumption for the cycle ergometer in the *ACSM's Guidelines for Exercise Testing and Prescription* (1995). These findings thus confirmed that the overall fitness of the subjects was poor and supported the inclusion criteria of the subjects prior to testing. Even though this study was designed to study rest and "moderate" exercise response at absolute workloads and a relative intensity of 60% of age-predicted maximum heart rate of untrained women, there may have been enough difference in fitness levels to cause problems in the comparisons of the group averages. Obtaining a peak VO_2 on subjects, and adding a relative intensity, *i.e.*, @ 60% peak VO_2, to the protocol might have provided more information. However, other studies have found that carbohydrate and fat utilization during exercise at 60% peak VO_2 were not influenced by levels of estrogen associated with menstrual phases (Kanaley, *et al.*, 1992).

There is no doubt that GnRHaa therapy does cause estrogen status to drop to menopausal levels, and even causes menopausal symptoms. It is, therefore, a good postmenopausal model. Also, since the estrogen drop is so large, it can be assumed that the results of this study are based on this predominant factor. However, it must also be considered to be a limitation of the study design because there may be inherent problems associated with endometriosis, for which the drug is administered, that may influence the results of this study. Moreover, the effects of the drug itself may influence results. Accounting for these influences, however, would be ethically impossible to measure. For instance, the effects of GnRHaa therapy could be studied only by adding back the normal level of estrogen after decreasing its synthesis. The administration of GnRHaa with concurrent full add-back estrogen, however, is not medically justified. Albeit an animal study may have some merit.

Taking into consideration that group M represented the low estrogen status and group F represented the high estrogen status during the menstrual cycle, it was surprising that group M did not demonstrate any significant differences from group F. Astrup and associates (1992), found that plasma estradiol concentration explained only 4% and 6% of the variation of 24 hour carbohydrate oxidation and lipid oxidation, respectively, during the follicular phase. Perhaps this variation was too small to be detected as significant in this study. However, there is at least one reason why the differences were not as pronounced as they could have been and relate to a design problem that must be discussed. Group F comprised both low and high estrogen levels. The mean estrogen level of group F was significantly

higher from the others, as expected, but the range overlapped considerably with group M. Even though no correlations were exhibited between estrogen, R, age and BMI in this study, a more defined picture of estrogen levels within the cycle could have been made by adding another group of late-follicular (pre-ovulatory) levels of estrogen to early follicular (menses) and mid-follicular levels (day 6-10). On the other hand, there is a question as to whether it is the absolute value of estrogen or the change in estrogen that is the important factor to consider. Also, some subjects may not have as wide a range of estrogen in their cycle. An alternate model that would eliminate within subject variability would be to test each subject at different phases of her menstrual cycle, and to test the GnRHaa group prior to beginning drug therapy and after two injections. However, this could not be done because of the possible confounding factor of pain that these subjects are experiencing prior to treatment.

Other possible reasons that could have influenced results are related to problems that were encountered in testing. The resting data collection was compromised, due to the equipment not measuring variables @ very low VE's, and this resulted in many missing values. Also, IV insertion may have caused variable responses, depending on how long it took to place the IV, and the subject's pain threshold. A waiting period of at least five minutes after placement would be warranted.

Finding no statistical differences among groups in the above metabolic and cardiorespiratory studies are corroborated by the results of blood assays. Analyses of blood lactate, cortisol, ammonia and glucose revealed no differences

among groups in their responses to increased workload from rest (0 minutes) to 10 minutes of exercise, to 20 minutes of exercise at steady state. These results are partially supported by those of Bonen (1983), Kanaley (1992) and McCracken (1994) and their respective associates. Apparently, estrogen status does not affect these parameters at either rest or at low-to-moderate intensity, short duration exercise. These parameters changed with intensity as any normal population would. Also, the lack of intergroup differences is not surprising since these factors do not change appreciably during only 20 minutes of submaximal intensity exercise. Twenty minutes is not enough time to notice a decrease in glucose (Pruett, 1971), or enough time or intensity to see depleted glycogen stores (Bergström, *et al.*, 1967). If there is no change in glucose utilization, indicating glycogen depletion, protein utilization, for the purpose of gluconeogenesis, would not be expected to increase (McArdle, *et al.*, 1991). Therefore, ammonia, the byproduct of protein catabolism, would be unchanged. In addition, lactate concentrations do not change even after 90 minutes of submaximal exercise (Issekutz, *et al.*, 1965). Finally, cortisol is released in response to high intensity or very prolonged exercise (Brooks, *et al.*, 1996), neither of which differed among groups.

Blood lipids were not significantly different among groups even though other studies have found differences between pre- and postmenopausal females (Rainville & Vaccaro, 1984). It is possible that group G had not been hypoestrogenic for a long enough period of time. The average time that the subjects were hypoestrogenic was approximately four weeks. On the other hand,

the study on the postmenopausal females, in the aforementioned study (Rainville & Vaccaro, 1984) had gone through natural menopause, and had not menstruated for at least a year prior to the experiment. Furthermore, studies of lipid metabolism have concluded that premenopausal cyclic changes in estrogen have only minor effects on lipid concentrations (Lebech, *et al.*, 1990) and FFA mobilization (Heiling & Jensen, 1992).

Based on all non-significant results, it is no surprise that crossover points for the three groups were not significantly different. However, there was a tendency towards the crossover points of the low estrogen group M and G to be reached sooner (30.80 and 32.85 watts, respectively) than that of group F (34.79 watts). This demonstrated that as intensity increased (and CHO utilization increased) the low estrogen groups came to 50% CHO more quickly than the highest estrogen group. These findings only corroborate, but cannot confirm, the present study's hypothesis, *i.e.*, that the pseudomenopausal estrogen status prefers carbohydrate to fat.

As in the resting analogy, the subtle metabolic differences found during exercise may have a long term effect on weight maintenance. For instance, if the results found at steady state were mainly attributed to estrogen status, as hypothesized, the tendency for carbohydrate utilization during moderate exercise might have a long term impact, just as seen in the previous resting analogy. Again, using the results of this study, of the fuel metabolized during steady state submaximal exercise, group F oxidized 63% carbohydrate and 37% fat (a 63:37 ratio), as compared to 70% and 30%, respectively, for group G (a 70:30 ratio). For

simplicity's sake, assume that total caloric expenditure during each exercise session is 200 kcal, and that this is done 5 times per week totaling 1000 kcal per week (4000 kcal per month). Premenopausal energy expenditure during exercise using the 63:37 ratio would be 2520:1480 kcal or 630:164 grams (1 g CHO = 4 kcal and 1 g fat = 9 kcal) per month. Postmenopausal values, using the 70:30 ratio would be 2800:1200 kcal or 700:133.3 grams. This represents a decrease of 280 kcal or 31 grams of fat oxidized per month, and extending to a year, 3348 kcal or 372 grams. Energy expenditure is the same, but there is a marked difference in the substrate oxidized. In other words, the calories expended are the same but they are not produced by the same substrate: a calorie is not a calorie. What are the long term consequences of this phenomenon? The preference of carbohydrate over fat should not affect body weight if all energy consumed is expended, but if the body is preferring to use CHO over fat, it is possible that protein/amino acid oxidation would occur (gluconeogenesis), and excess fat would be stored rather than used. It can be readily seen that the combined factors of CHO preference at rest and during submaximal exercise may result in weight gain. Under these circumstances, exercise may not counteract the weight gain that was shown to occur during resting metabolism. However, it certainly confirms the need and benefit to exercise regularly at a moderate level. These results are corroborated by a previous study performed by the author (Melton, *et al.*, 1996) using the rat model. Ovariectomy resulted in weight gain, and was only partially ameliorated by swim training.

Finally, trying to assess metabolic actions of a single hormone such as estrogen in an *in vivo* situation is a demanding task. Controlling for confounding factors is a major issue. In this study, there are at least two factors that could be limiting the present results. (1) Since body fat is positively correlated to estrogen levels, subjects with BMI's of >25 could have been excluded from the study; (2) Groups comprised of women with a very wide age range. Two decades difference seen in this study may have an unknown influence, i.e., some subjects were twice as old as other subjects. Separating the age groups into 21-31 and 32-42, therefore, would define differences if they exist.

CHAPTER 6. SUMMARY AND CONCLUSIONS

Clearly, estrogens have a major effect on the location and distribution of fat deposition in the body, as shown at puberty, when females begin to develop breasts, and to increase the size of the buttocks and thighs. After puberty, estrogens have an important role in fertility and maintaining the hormonal milieu during the childbearing years. At the end of the fertile cycle, the female experiences menopause, and estrogen levels decline to that of normal male values. During this time weight gain is a common occurrence (Wing, *et al*, 1991) and may be caused by a variety of factors. Since the weight gain is associated with chronically low estrogen status, there may be a link to the fuel mixture being used by the postmenopausal female, specifically, that of a higher ratio of carbohydrate to fat oxidation than what is used by premenopausal females. If carbohydrate is being utilized preferentially, the unused fat would be delivered to storage, thus increasing fat cell size and causing weight gain. Previous studies have demonstrated that estrogen status during phases of the menstrual cycle have been associated with fuel substrate utilization changes. The lack of estrogen, or low estrogen status, during the menopause has also been associated with lipid and glucose oxidation differences. The present study, used eumenhorreic subjects for comparison and GnRHaa-induced amenohorreic subjects as a postmenopausal model and determined fuel substrate utilization by indirect calorimetry. Whereas the results suggested possible differences due to estrogen status, these differences were not conclusive.

Subjects in group M, with acutely low estrogen status (several days), did not generally respond in a similar fashion as the subjects in group G, who had chronically low estrogen status (a month or more). Keeping in mind that estrogen, as a steroid hormone, meets its receptor in the nucleus of the cell and activates the expression of genetic systems. This process takes a longer time to employ than those hormone-receptor systems that act immediately, *e.g.,* insulin which meets its receptor on the target cell membrane activating glucose transport into the cell simultaneously. Subjects in group M, therefore, may not have had enough time to demonstrate the same results as those in group G, and may answer the question that Bunt (1990) raised: "*...it is not known what is more relevant for acute exercise responses: chronic E_2 status or E_2 levels just prior to exercise*?".

Ambiguities still exist, however, and further research is necessary in order to more clearly define the trends that were demonstrated in this study. For example, one study that would eliminate the time factor difference between postmenopausal estrogen values mentioned above, using the GnRHaa model, test at one week after postmenopausal values have been established in order to mimic menses, and compare the results after 3 to 6 months of therapy.

Another ambiguity is the interaction of diet and estrogen status, and how these two affect fuel substrate utilization. A possible experiment may be set up to use the same three groups as this study, except limiting the follicular to pre-ovulatory (highest estrogen status). The subjects would be placed on a controlled diet of high fat or high carbohydrate for a week. Using a metabolic chamber for a 24 hour period, the subjects could be tested for basal as well as exercise energy

expenditure and carbohydrate and fat oxidation rates. If 24 hour urine were collected, protein utilization could also be established. It could be established that the postmenopausal female needs to increase her carbohydrate intake and reduce her fat intake in order to counter the body's decrease in fat utilization and subsequent increase in fat storage.

Finally, the GnRHaa model could be used in an exercise training study. There are many ways to study training effects, but the caloric cost of training is an important aspect with respect to menopausal status. Is a calorie a calorie? In other words, is the postmenopausal female going to illicit the same benefits as a premenopausal female even if she is not using fat as readily? Investigating the long term effects of low estrogen status on fuel metabolism during a longitudinal training study would verify the calculations of the previous chapter.

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APPENDIX A. CARDIORESPIRATORY, METABOLIC, AND WORK DATA OF SUBJECTS

Table A.1. Average R Values at Rest per Subject.

Subject	Group M	Group F	Group G
1	0.88± 0.05	0.79±0.05	0.88±0.04
2	0.87± 0.03	0.91±0.09	0.82±0.05
3	0.88± 0.05	0.77± 0.08	0.79±0.06
4	0.90± 0.15	0.93± 0.10	0.85±0.10
5	0.94± 0.06	0.90 ±0.06	0.87±0.08
6	0.74± 0.04	0.85 ±0.02	1.03±0.13
7	0.94± 0.06	0.95± 0.08	0.97±0.09
8	0.89± 0.05	0.89± 0.14	
9	0.85± 0.04	0.81± 0.05	
10	0.81± 0.06	0.98± 0.11	
11	0.96± 0.07	0.87± 0.07	
12			
13			
14			
Total Group	0.883± 0.06	0.891 ± 0.07	0.89 ±0.08

Values are mean ± standard deviation.
No significant differences among group means.

Table A.2. Absolute and Relative Oxygen Consumption: VO_2 (L/min) and VO_2 (ml/kg/min) STPD of Subjects at Rest.

Subject	Group M		Group F		Group G	
	L/min	ml/kg/min	L/min	ml/kg/min	L/min	ml/kg/min
1	0.11±0.07	2.0±1.2	0.14±0.05	2.4±0.9	0.09±0.02	1.2±0.3
2	0.14±0.02	2.3±0.4	0.11±0.05	1.8±0.9	0.12±0.04	1.8±0.8
3	0.22±0.04	3.2±0.5	0.18±0.13	2.1±2.0	0.19±0.06	3.4±1.1
4	0.10±0.02	1.7±0.4	0.16±0.06	1.4±1.0	0.11±0.02	1.7±0.5
5	0.15±0.04	3.0±0.9	0.17±0.11	2.8±2.1	0.11±0.02	1.1±0.4
6	0.21±0.05	3.3±0.8	0.09±0.01	1.2±0.1	0.07±0.01	1.4±0.3
7	0.17±0.10	2.6±1.7	0.13±0.02	2.2±0.4	0.13±0.06	1.6±0.8
8	0.17±0.11	2.5±1.9	0.14±0.09	2.7±1.8		
9	0.14±0.04	2.3±0.9	0.12±0.04	2.1±0.8		
10	0.14±0.04	2.6±0.8	0.12±0.02	2.4±0.4		
11	0.13±0.02	2.0±0.4	0.23±0.08	2.3±1.7		
12			0.13±0.04	1.8±0.9		
Group	0.15±0.03	2.5±0.5	0.14±0.03	2.1±0.4	0.12±0.03	1.7±0.7 [a]

Values are mean ± standard deviation.
[a] Different from Group M ($p = 0.0395$)

Table A.3. Carbon Dioxide Production (VCO_2 in L/min) of Subjects at Rest.

Subject	Group M	Group F	Group G
1	0.10±0.06	0.11±0.04	0.08±0.02
2	0.13±0.02	0.10±0.05	0.10±0.03
3	0.19±0.03	0.15±0.13	0.15±0.04
4	0.09±0.03	0.15±0.07	0.09±0.02
5	0.14±0.05	0.16±0.11	0.10±0.02
6	0.15±0.04	0.07±0.00	0.08±0.01
7	0.17±0.10	0.12±0.03	0.13±0.07
8	0.15±0.11	0.12±0.06	
9	0.12±0.04	0.09±0.03	
10	0.11±0.04	0.12±0.02	
11	0.13±0.02	0.23±0.10	
12		0.11±0.04	
Group	0.14±0.02	0.13±0.04	0.10± 0.02

Values are mean ± standard deviation.
No significant differences among group means.

Table A.4. Average VE (BTPS) at Rest (Liters/minute).

Subject	Group M	Group F	Group G
1	1.06±0.9	3.41±0.8	5.14±08
2	5.32±0.8	2.39±1.5	2.14±1.3
3	6.77±1.0	4.11±1.9	5.44±1.6
4	4.81±1.5	2.15±1.4	2.64±1.3
5	2.60±1.4	1.87±1.2	1.76±0.6
6	6.35±1.2	4.69±0.6	1.88±0.8
7	2.89±1.7	4.62±1.2	2.21±1.9
8	4.03±3.7	2.65±1.1	
9	3.02±1.1	2.77±1.0	
10	5.11±1.3	6.2±1.2	
11	3.10±1.2	3.2±2.3	
12		1.7±1.2	
13			
14			
Group	4.10±1.7	3.33±1.36	3.07±1.5

Values are mean ± standard deviation.
No significant differences among group means.

Table A.5. Average Heart Rate (HR) and Percent Age-predicted Maximum Heart Rate (%HR) of Subjects at Rest.

Subject	Group M HR(BPM)	%HR	Group F HR (BPM)	%HR	Group G HR (BPM)	%HR
1	86.3±5.6	45.5±2.9	80.4±2.3 43.1±1.3		73.7±5.4	38.7±2.8
2	88.7±2.7	46.5±1.5	75.7±3.4 38.5±1.8		56.8±2.1	29.5±1.1
3	66.8±3.3	35.3±1.7	83.4±5.5 42.9±2.9		69.9±2.5	35.8±1.3
4	74.4±2.8	40.2±1.5	61.7±2.2 33.4±1.3		63.2±2.2	32.5±1.1
5	73.7±2.0	38.1±1.0	75.4±2.2 38.3±1.2		61.2±2.4	31.9±1.2
6	63.2 ± 2.8	34.4±2.4	88.7±3.1 47.3±1.7		50.6±4.8	26.9±2.7
7	67.1±3.1	35.2±1.5				
8	71.5±2.5	38.6±1.3				
9	77.2±1.7	41.3±0.8				
10	71.0±3.0	36.1±1.5				
11						
12						
13						
14						
Group	74.0±8.2	39.1±4.2	76.0±10.3 39.8±5.5		62.6±8.4 [a]	32.5±4.2 [b]

Values are mean ± standard deviation.

[a] Different from Group F ($p = 0.0415$) and Group M ($p = 0.0549$)
[b] Different from Group F ($p = 0.0344$) and Group M ($p = 0.0332$)

Table A.6. Percent Carbohydrate Used as a Fuel Substrate at Rest.

Subject	Group M	Group F	Group G
1	61.9	31.4	63.5
2	42.6	71.3	58.8
3	31.2	25.6	61.2
4	54.3	79.6	68.7
5	69.5	68.3	81.5
6	100.0	52.4	14.1
7	92.2	88.2	81.0
8		63.1	65.9
9		34.5	52.8
10		100.0	40.1
11		95.2	89.4
12		59.1	
13			
14			
Group±SD	64.9±21.03	64.5±24.76	61.8±25.00

No significant differences among group means.

Table A.7. Kilocalories Expended Per Minute at Rest.

Subject	Group M	Group F	Group G
1	0.62±1.06	0.72±0.45	0.23±0.42
2	0.96±0.19	0.33±0.48	0.73±0.45
3	0.42±0.50	0.80±0.83	1.03±0.19
4	0.88±0.33	0.77±0.66	0.66±0.48
5	1.03±0.19	0.88±0.32	0.14±0.36
6	1.00±0.65	0.63±0.58	0.54±0.52
7	0.94±0.24	0.96±0.19	
8	0.85±0.36	1.33±0.51	
9		0.75±0.46	
10			
11			
12			
13			
14			
Group	0.78±0.17	0.72±0.19	0.61±0.17

Values are mean ± standard deviation.
No significant differences among group means.

Table A.8. Kilocalories Expended per Liter Oxygen at Rest.

Subject	Group M	Group F	Group G
1	4.75±0.10	4.77±0.07	4.89±0.06
2	4.88±0.04	4.87±0.12	4.76±0.09
3	4.90±0.06	4.70±0.05	4.79±0.07
4	4.88±0.12	4.74±0.12	4.80±0.12
5	4.86±0.14	4.76±0.12	4.71±0.08
6	4.73±0.04	4.77±0.09	4.85±0.16
7	4.86±0.14	4.96±0.08	4.80±0.15
8	4.81±0.12	4.84±0.12	
9	4.79±0.09	4.78±0.08	
10	4.82±0.08	5.01±0.05	
11	4.85±0.15	4.75±0.12	
12		4.74±0.10	
13			
14			
Group	4.90±0.08	4.91±0.09	4.91±0.09

Values are mean ± standard deviation.
No significant differences among group means.

TABLE A.9. Resting Energy Expenditure (kcal/day).

Subject	Group M	Group F	Group G
1	1181.38±1255.02	985.09±354.93	675.26±170.24
2	1072.93±194.59	756.66±323.64	857.93±294.51
3	764.15±182.85	1280.40±985.89	1312.59±407.91
4	1110.71±365.27	1306.67±865.78	785.00±202.94
5	1459.15±328.97	995.53±238.98	577.50±125.30
6	1209.53±849.31	1005.00±639.45	954.09±447.86
7	1104.35±362.62	927.92±178.63	
8	1013.22±293.23	1671.00±596.13	
9		996.50±326.13	
10			
11			
12			
13			
14			
Group	1122.00±247.68	1039.81±270.77	871.28±240.56

Values are mean ± standard deviation.
No significant differences among group means.

Table A.10. Average R Values* of Subjects and Groups at 0, 10, 20, 30 and 40 Watts.

Subject	Watts	Group M	Group F	Group G
1	0	0.8800	0.7054	0.7950
	10	0.8700	0.7100	0.8200
	20	0.9000	0.7700	0.8150
	30	0.9250	0.8050	0.8450
	40	1.0050	0.8050	0.8900
2	0	0.7300	0.7100	0.7050
	10	0.8050	0.7800	0.7600
	20	0.8350	.	0.7850
	30	0.9200	0.8250	0.8050
	40	0.9900	0.8500	0.8550
3	0	0.7450	.	0.7700
	10	0.8150	.	0.7650
	20	0.8400	0.8300	0.7450
	30	0.9000	0.8650	0.8100
	40	0.9750	0.9450	0.8450
4	0	0.7700	0.6800	0.7900
	10	0.8000	0.7500	0.7450
	20	0.8300	0.8150	0.8350
	30	0.8350	0.8750	0.8650
	40	0.8850	0.9650	0.8950
5	0	0.8500	0.7800	0.8050
	10	0.8750	0.8000	0.8150
	20	0.8900	0.8050	0.8150
	30	0.9900	0.8200	0.8750
	40	1.055	0.8900	0.8900
6	0	0.6950	0.7800	0.7700
	10	0.7400	0.7800	0.8000
	20	0.7600	0.7600	0.8500
	30	0.7850	0.7900	0.8250
	40	0.8550	0.8150	0.8850
7	0	0.7550	0.6800	0.8550
	10	0.7900	0.8200	0.9000
	20	0.8150	0.9100	0.8050
	30	0.8200	0.9500	0.8050
	40	0.8450	1.0400	0.8750
8	0	0.7100	0.6900	
	10	0.7900	0.7700	
	20	0.8150	0.7550	
	30	0.9000	0.7850	
	40	0.9450	0.8400	
9	0	0.7150	0.7600	
	10	0.7850	0.8200	
	20	0.8000	0.8100	
	30	0.8100	0.7800	
	40	0.8050	0.8450	

* Values are average of last two 30-second periods of each two minute stage.
No Significant Differences among group means.
(Table cont'd)

Subject	Watts	Group M	Group F	Group G
10	0	0.7900	0.7500	
	10	0.8300	0.7450	
	20	0.8350	0.8150	
	30	0.8500	0.9350	
	40	0.8650	0.9950	
11	0		0.7600	
	10		0.8400	
	20		0.7950	
	30		0.9000	
	40		0.9850	
12	0		0.7900	
	10		0.7750	
	20		0.7350	
	30		0.8100	
	40		0.8700	
13	0		0.8700	
	10		0.8800	
	20		0.9000	
	30		0.9050	
	40		0.9300	
14	0		0.8050	
	10		0.8700	
	20		0.8450	
	30		0.8900	
	40		0.9000	
Group Means ± SD	0	0.7640 ± 0.06	0.7525 ± 0.06	0.7842 ± 0.05
	10	0.8100 ± 0.04	0.7969 ± 0.05	0.7930 ± 0.04
	20	0.8320 ± 0.04	0.8072 ± 0.05	0.8071 ± 0.03
	30	0.8735 ± 0.06	0.8488 ± 0.05	0.8328 ± 0.03
	40	0.9225 ± 0.08	0.9003 ± 0.07	0.9225 ± 0.03

Table A.11. Average Absolute (L) and Relative (ml/kg/min) Oxygen Consumption Values[a] of Subjects at 0, 10, 20, 30 and 40 Watts.

Subject	Watts	Group M		Group F		Group G	
		L	ml/kg/min	L	ml/kg/min	L	ml/kg/min
1	0	0.21	2.7	0.32	5.6	0.31	4.0
	10	0.22	3.2	0.30	5.3	0.31	4.0
	20	0.30	4.0	0.31	5.5	0.35	4.5
	30	0.34	5.5	0.37	6.5	0.42	5.4
	40	0.50	7.9	0.47	8.3	0.56	7.3
2	0	0.28	3.3	0.28	4.6	0.24	4.4
	10	0.23	3.5	0.30	4.9	0.23	4.2
	20	0.25	4.8	3	.	0.30	5.4
	30	0.34	5.4	0.41	6.7	0.37	6.7
	40	0.44	7.9	0.57	9.3	0.46	8.4
3	0	0.18	4.0	.	.	0.23	4.0
	10	0.21	3.3	.	1.1	0.30	5.5
	20	0.27	3.6	0.27	4.2	0.26	4.6
	30	0.32	4.9	0.26	4.2	0.29	5.1
	40	0.42	6.2	0.42	6.8	0.43	7.7
4	0	0.26	2.9	0.17	2.8	0.27	4.3
	10	0.24	3.4	0.36	.	0.30	4.8
	20	0.29	4.2	0.30	5.2	0.38	6.1
	30	0.43	5.0	0.35	5.9	0.49	7.8
	40	0.62	6.6	0.54	9.2	0.64	10.3
5	0	0.30	5.0	0.25	3.6	0.25	3.4
	10	0.34	4.7	0.27	3.9	0.30	4.0
	20	0.34	5.7	0.33	4.8	0.42	5.6
	30	0.44	8.4	0.53	7.6	0.44	6.0
	40	0.55	12.0	0.60	8.6	0.58	7.8
6	0	0.20	4.7	0.37	4.0	0.25	4.5
	10	0.28	5.5	0.14	2.6	0.19	3.4
	20	0.30	5.3	0.16	2.9	0.26	4.8
	30	0.43	7.0	0.22	4.1	0.28	5.0
	40	0.50	8.7	0.36	6.6	0.32	5.8
7	0	0.19	3.3	0.23	3.3	0.18	2.4
	10	0.23	4.7	0.18	2.6	0.14	1.4
	20	0.31	5.0	0.23	3.2	0.21	2.8
	30	0.40	7.1	0.28	3.9	0.21	2.7
	40	0.55	8.2	0.39	5.5	0.28	3.9
8	0	0.22	3.2	0.25	3.3		
	10	0.27	4.0	0.23	3.0		
	20	0.31	5.3	0.24	3.2		
	30	0.39	6.8	0.42	5.5		
	40	0.52	9.4	0.59	7.7		
9	0	0.30	3.9	0.30	5.0		
	10	0.36	4.9	0.30	4.9		
	20	0.40	5.6	0.36	6.1		
	30	0.48	7.0	0.43	7.2		
	40	0.65	9.4	0.59	9.8		

[a]Values are average of last two 30-second periods of each two minute stage.
No Significant Differences among group means.
(Table cont'd)*

Subject	Watts	Group M		Group F		Group G	
		L	ml/kg/min	L	ml/kg/min	L	ml/kg/min
10	0	0.16	4.5	0.22	4.4		
	10	0.18	5.4	0.25	5.1		
	20	0.23	6.1	0.35	7.0		
	30	0.31	7.2	0.38	7.6		
	40	0.45	9.8	0.53	10.6		
11	0			0.15	2.8		
	10			0.21	4.0		
	20			0.27	5.0		
	30			0.36	6.8		
	40			0.53	10.0		
12	0			0.17	3.2		
	10			0.20	4.0		
	20			0.19	3.6		
	30			0.27	5.2		
	40			0.42	8.0		
13	0			0.33	5.4		
	10			0.35	5.7		
	20			0.39	6.5		
	30			0.44	7.3		
	40			0.54	9.1		
14	0			0.30	5.3		
	10			0.25	4.4		
	20			0.35	6.2		
	30			0.38	6.8		
	40			0.49	8.6		
Group Means ± SD	0	0.22±0.05	3.7±0.8	0.25±0.06	4.1±1.3	0.24±0.04	3.8±0.8
	10	0.25±0.06	4.2±1.0	0.26±0.06	7.7±17.6	0.26±0.06	3.9±1.4
	20	0.30±0.05	4.9±0.9	0.28±0.07	4.9±1.3	0.31±0.07	4.8±1.1
	30	0.38±0.05	6.4±1.1	0.36±0.08	6.1±1.3	0.35±0.10	5.5±1.5
	40	0.51±0.07	8.6±1.6	0.50±0.08	8.5±1.4	0.46±0.13	7.3±1.9

Table A.12. Average Carbon Dioxide Production Values[a] (L/min) of Subjects at 0, 10, 20, 30 and 40 Watts.

Subject	Watts	Group M	Group F	Group G
1	0	0.14	0.22	0.24
	10	0.16	0.22	0.26
	20	0.21	0.24	0.29
	30	0.29	0.30	0.35
	40	0.45	0.37	0.50
2	0	0.16	0.20	0.17
	10	0.18	0.23	0.18
	20	0.25	.	0.24
	30	0.31	0.34	0.30
	40	0.50	0.49	0.40
3	0	0.21	.	0.18
	10	0.19	.	0.24
	20	0.21	0.22	0.19
	30	0.31	0.23	0.24
	40	0.42	0.40	0.36
4	0	0.14	0.12	0.21
	10	0.17	0.27	0.22
	20	0.22	0.25	0.32
	30	0.27	0.30	0.42
	40	0.37	0.52	0.57
5	0	0.22	0.20	0.20
	10	0.21	0.22	0.24
	20	0.26	0.27	0.34
	30	0.43	0.43	0.39
	40	0.65	0.54	0.52
6	0	0.20	0.29	0.19
	10	0.25	0.11	0.15
	20	0.25	0.12	0.23
	30	0.34	0.17	0.23
	40	0.47	0.29	0.28
7	0	0.15	0.16	0.15
	10	0.22	0.15	0.13
	20	0.25	0.21	0.16
	30	0.35	0.26	0.17
	40	0.42	0.41	0.25
8	0	0.13	0.18	
	10	0.18	0.18	
	20	0.25	0.19	
	30	0.36	0.33	
	40	0.52	0.49	
9	0	0.16	0.23	
	10	0.21	0.24	
	20	0.25	0.30	
	30	0.31	0.33	
	40	0.42	0.50	

[a] Values are average of last two 30-second periods of each two minute stage.
No Significant Differences among group means.
(Table cont'd)

Subject	Watts	Group M	Group F	Group G
10	0	0.23	0.17	
	10	0.30	0.19	
	20	0.33	0.29	
	30	0.41	0.36	
	40	0.56	0.53	
11	0		0.12	
	10		0.18	
	20		0.22	
	30		0.33	
	40		0.53	
12	0		0.13	
	10		0.16	
	20		0.16	
	30		0.22	
	40		0.36	
13	0		0.29	
	10		0.30	
	20		0.35	
	30		0.40	
	40		0.51	
14	0		0.24	
	10		0.21	
	20		0.30	
	30		0.34	
	40		0.44	
Group Means ± SD	0	0.17±0.04	0.19±0.05	0.19±0.03
	10	0.20±0.05	0.20±0.05	0.20±0.05
	20	0.24±0.04	0.23±0.06	0.25±0.06
	30	0.33±0.05	0.31±0.07	0.29±0.09
	40	0.47±0.08	0.45±0.08	0.40±0.12

Table A.13. Average Minute Ventilation (L/min) Values[a] of Subjects and Groups at 0, 10, 20, 30 and 40 Watts.

Subject	Watts	Group M	Group F	Group G
1	0	4.1	5.3	10.0
	10	4.9	6.7	10.5
	20	5.9	8.8	11.1
	30	8.8	10.7	12.5
	40	13.6	13.1	16.9
2	0	5.3	5.3	3.1
	10	6.0	7.7	3.5
	20	7.5	.	6.2
	30	9.6	10.6	8.1
	40	15.8	13.9	11.1
3	0	3.4	0.4	4.1
	10	4.9	2.5	4.5
	20	6.3	3.6	6.1
	30	9.1	6.7	7.7
	40	13.3	12.8	11.1
4	0	5.3	5.4	7.9
	10	6.7	7.8	6.5
	20	9.3	9.8	9.9
	30	11.4	12.7	13.4
	40	14.7	17.2	16.6
5	0	8.1	6.1	5.2
	10	8.4	7.1	7.2
	20	10.4	8.5	9.1
	30	16.0	12.3	10.3
	40	22.8	15.3	13.6
6	0	7.8	2.0	3.1
	10	9.2	1.6	2.3
	20	9.9	3.0	5.1
	30	13.2	5.2	6.6
	40	17.3	8.3	8.8
7	0	4.2	4.0	2.5
	10	5.4	7.0	3.0
	20	8.0	9.0	2.6
	30	11.1	1.0	4.1
	40	12.2	15.0	6.6
8	0	3.8	3.5	
	10	6.1	4.7	
	20	8.2	5.1	
	30	11.2	11.3	
	40	15.2	15.9	
9	0	5.4	7.8	
	10	7.3	8.7	
	20	9.2	11.3	
	30	11.1	11.8	
	40	13.2	16.6	

[a]Values are average of last two 30-second periods of each two minute stage
[b]Different from Group M (p=0.0684)
(Table cont'd)

Subject	Watts	Group M	Group F	Group G
10	0	8.3	5.3	
	10	9.8	5.7	
	20	11.1	7.4	
	30	13.4	10.5	
	40	17.5	12.5	
11	0		2.6	
	10		5.1	
	20		5.7	
	30		10.1	
	40		14.8	
12	0		4.6	
	10		4.7	
	20		4.2	
	30		6.6	
	40		10.3	
13	0		7.2	
	10		7.8	
	20		8.7	
	30		9.5	
	40		11.8	
14	0		5.9	
	10		6.1	
	20		8.0	
	30		10.1	
	40		13.2	
Group Means ± SD	0	5.58±1.84	4.71±2.11	5.13±2.77
	10	5.91±2.23	6.89±1.79	5.37±2.89
	20	8.59±1.78	7.09±2.66	7.14±2.92
	30	11.50±2.25	9.91±2.43	9.00±3.24
	40	15.56±3.23	13.60±2.92	12.12±3.75[b]

Table A.14. Average HR and Percent Age-predicted HR maximum (%HR) Values[a] of Subjects at 0, 10, 20, 30 and 40 Watts.

Subject	Watts	Group M		Group F		Group G	
		HR	%HR	HR	%HR	HR	%HR
1	0	107	57	85	46	101	52
	10	112	59	88	47	102	53
	20	114	60	89	48	109	56
	30	121	64	99	53	120	62
	40	138	73	107	58	123	64
2	0	98	51	92	50	83	44
	10	103	54	96	53	83	44
	20	107	56	98	.	91	48
	30	114	60	103	56	87	51
	40	124	66	114	62	106	56
3	0	86	46	90	48	71	37
	10	91	48	95	51	74	39
	20	95	50	97	52	80	42
	30	106	56	104	56	86	44
	40	113	60	.	.	97	50
4	0	.	.	79	43	88	45
	10	100	52	94	51	93	48
	20	104	54	99	54	101	52
	30	115	60	109	59	108	56
	40	126	66	120	65	117	61
5	0	90	49	103	53	82	42
	10	93	50	110	56	86	44
	20	95	52	118	60	94	48
	30	106	58	126	65	98	50
	40	118	64	142	73	104	54
6	0	86	44	83	45	88	46
	10	90	47	86	47	90	47
	20	86	49	90	49	94	49
	30	106	54	105	57	105	55
	40	112	58	112	61	117	62
7	0	81	44	87	48	78	42
	10	86	46	89	49	81	43
	20	91	49	98	54	85	45
	30	97	52	108	60	90	48
	40	107	58	123	68	99	53
8	0	90	47	87	44		
	10	95	50	92	47		
	20	103	54	105	54		
	30	115	60	119	61		
	40	133	70	135	69		

[a] Values are average of last two 30-second periods of each two minute stage
[b] Different from Group F (p = 0.0525)
(Table cont'd)

Subject	Watts	Group M		Group F		Group G	
		HR	%HR	HR	%HR	HR	%HR
9	0	84	46	101	52		
	10	87	47	107	55		
	20	90	49	108	55		
	30	98	53	114	58		
	40	105	57	126	64		
10	0	84	43	94	49		
	10	90	46	99	51		
	20	93	48	106	54		
	30	98	50	116	60		
	40	109	56	126	65		
11	0			79	43		
	10			81	44		
	20			86	46		
	30			94	51		
	40			107	58		
12	0			89	45		
	10			93	47		
	20			99	51		
	30			107	54		
	40			114	59		
13	0			85	44		
	10			88	45		
	20			86	44		
	30			92	47		
	40			99	50		
14	0			104	56		
	10			107	57		
	20			112	60		
	30			118	63		
	40			125	67		
Group Means ± SD	0	89.3 ±8.0	47.2 ±4.2	90.2 ±8.5	47.6 ±4.1	82.9 ±8.4	43.2 ±4.2
	10	94.6 ±9.3	49.8 ±4.4	94.6 ±9.3	49.8 ±4.4	85.7 ±8.0	44.6 ±4.0
	20	98.2 ±8.0	51.8 ±4.0	98.5 ±10.3	51.6 ±5.0	91.9 ±8.5	47.8 ±4.1
	30	106.8 ±8.2	56.2 ±4.2	106.5±9.7	56.1 ±5.0	98.6 ±10.1	51.3 ±5.1
	40	117.8 ±11.3	62.0 ±6.0	116.5 ±11.2	61.3 ±5.8	107.6 ±9.3	56.2 ±4.8

Table A.15. Average Percent Carbohydrate Values[a] of Subjects at 0, 10, 20, 30 and 40 Watts.

Subject	Watts	Group M	Group F	Group G
1	0	60.81	4.20	31.61
	10	57.45	2.38	40.33
	20	67.51	22.74	38.58
	30	75.78	35.06	48.93
	40	100.00	35.11	64.10
2	0	8.39	6.01	4.20
	10	35.09	26.31	19.17
	20	45.51	0	28.09
	30	74.01	41.96	35.11
	40	96.81	50.65	52.36
3	0	13.71	0	22.69
	10	38.54	0	20.99
	20	47.22	43.79	13.82
	30	67.51	55.76	36.85
	40	92.01	82.31	48.94
4	0	23.61	0	29.85
	10	33.36	7.81	13.80
	20	43.71	38.42	45.46
	30	45.51	59.12	55.65
	40	62.48	88.76	65.74
5	0	50.65	26.32	35.09
	10	59.14	33.28	38.58
	20	64.17	35.11	38.58
	30	95.21	40.25	59.14
	40	100.00	64.17	64.04
6	0	0	13.16	22.44
	10	12.01	13.16	33.36
	20	19.20	19.13	50.62
	30	28.07	29.85	41.96
	40	52.36	38.58	62.50
7	0	17.36	0	52.19
	10	29.77	20.16	33.75
	20	38.60	35.41	35.00
	30	40.33	41.97	35.09
	40	48.89	50.00	59.14
8	0	2.38	0	
	10	29.84	22.69	
	20	38.58	17.40	
	30	67.51	27.98	
	40	82.33	47.23	
9	0	2.93	19.17	
	10	28.09	40.25	
	20	33.36	36.72	
	30	36.85	26.31	
	40	35.01	48.94	

[a]Values are average of last two 30-second periods of each two minute stage.
No Significant Differences among group means.
(Table cont'd)

Subject	Watts	Group M	Group F	Group G
10	0	29.85	18.43	
	10	43.79	13.80	
	20	45.51	38.60	
	30	50.66	79.01	
	40	55.70	88.71	
11	0		19.20	
	10		47.22	
	20		31.61	
	30		67.51	
	40		95.21	
12	0		29.84	
	10		24.49	
	20		10.19	
	30		36.85	
	40		57.42	
13	0		57.31	
	10		60.81	
	20		67.51	
	30		69.07	
	40		77.39	
14	0		34.74	
	10		57.44	
	20		48.93	
	30		64.17	
	40		67.51	
Group Means ± SD	0	20.97 ±22.3	16.31 ±19.2	28.30 ±18.1
	10	36.71 ±14.6	26.42 ±21.0	28.57 ±17.1
	20	44.34 ±14.3	31.82 ±20.6	35.74 ±13.6
	30	58.14 ±21.1	48.21 ±21.7	44.68 ±12.1
	40	72.56 ±24.4	63.71 ±24.3	59.55 ±10.23

Table A.16. Average* Kilocalories Expended Per Liter of Oxygen of Subjects at 0, 10, 20, 30 and 40 Watts.

Subject	Watts	Group M	Group F	Group G
1	0	4.89	4.70	4.79
	10	4.88	4.69	4.82
	20	4.92	4.76	4.81
	30	4.95	4.80	4.85
	40	5.04	4.80	4.91
2	0	4.71	4.70	4.70
	10	4.80	4.77	4.75
	20	4.84	4.68	4.78
	30	4.94	4.83	4.80
	40	5.03	4.86	4.86
3	0	4.73	4.68	4.76
	10	4.81	4.68	4.75
	20	4.84	4.83	4.73
	30	4.92	4.88	4.81
	40	5.01	4.97	4.85
4	0	4.76	4.68	4.78
	10	4.80	4.71	4.73
	20	4.83	4.81	4.84
	30	4.84	4.89	4.88
	40	4.90	5.00	4.91
5	0	4.86	4.77	4.80
	10	4.89	4.80	4.81
	20	4.91	4.80	4.81
	30	5.02	4.82	4.89
	40	5.04	4.91	4.91
6	0	4.68	4.73	4.76
	10	4.72	4.73	4.80
	20	4.75	4.75	4.86
	30	4.78	4.78	4.83
	40	4.86	4.81	4.90
7	0	4.74	4.68	4.86
	10	4.78	4.75	4.80
	20	4.81	4.81	4.80
	30	4.82	4.83	4.80
	40	4.85	4.86	4.89
8	0	4.69	4.68	
	10	4.78	4.76	
	20	4.81	4.74	
	30	4.92	4.78	
	40	4.97	4.84	
9	0	4.69	4.75	
	10	4.78	4.82	
	20	4.80	4.81	
	30	4.81	4.77	
	40	4.80	4.85	

*Values are average of last two 30-second periods of each two minute stage.
No Significant Differences among group means.
(Table cont'd)

Subject	Watts	Group M	Group F	Group G
10	0	4.78	4.74	
	10	4.83	4.73	
	20	4.84	4.81	
	30	4.86	4.96	
	40	4.88	5.00	
11	0		4.75	
	10		4.84	
	20		4.79	
	30		4.92	
	40		5.02	
12	0		4.78	
	10		4.76	
	20		4.72	
	30		4.81	
	40		4.88	
13	0		4.88	
	10		4.89	
	20		4.92	
	30		4.92	
	40		4.96	
14	0		4.80	
	10		4.88	
	20		4.85	
	30		4.91	
	40		4.92	
Group Means ± SD	0	4.75±0.07	4.74±0.06	4.78±0.06
	10	4.81±0.05	4.77±0.07	4.78±0.06
	20	4.84±0.05	4.79±0.07	4.80±0.04
	30	4.89±0.07	4.85±0.07	4.84±0.04
	40	4.94±0.09	4.91±0.08	4.89±0.03

Table A.17. Average[a] Kilocalories Expended Per Minute at 0, 10, 20, 30 and 40 Watts.

Subject	Watts	Group M	Group F	Group G
1	0	1.0	1.0	1.5
	10	1.0	1.5	1.5
	20	1.0	1.5	2.0
	30	1.5	2.0	2.0
	40	2.0	2.0	3.0
2	0	1.0	1.0	1.0
	10	1.0	1.5	1.0
	20	1.5	.	1.5
	30	2.0	2.0	2.0
	40	2.5	3.0	2.0
3	0	1.5	.	1.0
	10	1.0	.	1.5
	20	1.0	1.0	1.0
	30	2.0	1.0	1.0
	40	2.0	2.0	2.0
4	0	1.0	1.0	1.0
	10	1.0	2.0	1.5
	20	1.0	1.5	2.0
	30	1.5	2.0	2.0
	40	2.0	2.5	3.0
5	0	1.0	1.0	1.0
	10	1.0	1.0	1.5
	20	1.5	2.0	2.0
	30	2.0	3.0	2.0
	40	3.0	3.0	3.0
6	0	1.0	2.0	1.0
	10	1.5	1.0	1.0
	20	2.0	1.0	1.0
	30	2.0	1.0	1.0
	40	3.0	2.0	2.0
7	0	1.0	.	1.0
	10	1.0	.	1.0
	20	1.5	.	1.0
	30	2.0	.	1.0
	40	2.0	.	1.0
8	0	1.0	1.0	
	10	1.0	1.0	
	20	1.5	1.0	
	30	2.0	2.0	
	40	3.0	3.0	
9	0	1.0	.	
	10	1.0	.	
	20	1.5	.	
	30	2.0	.	
	40	2.5	.	

[a] Values are average of last two 30-second periods of each two minute stage
No significant differences among group means
(Table cont'd)

Subject	Watts	Group M	Group F	Group G
10	0 10 20 30 40	1.0 2.0 2.0 2.0 3.0	1.0 1.0 1.5 2.0 3.0	
11	0 10 20 30 40		. . . . .	
12	0 10 20 30 40		1.0 1.0 1.0 1.0 2.0	
13	0 10 20 30 40		1.5 2.0 2.0 2.0 3.0	
14	0 10 20 30 40		1.5 1.0 2.0 2.0 2.5	
Group Means ± SD	0 10 20 30 40	1.05 ±0.22 1.15 ±0.36 1.45 ±0.51 1.90 ±0.30 2.5 ±0.51	1.15 ± 0.37 1.27 ±0.46 1.45 ±0.51 1.81 ±0.58 2.54 ±0.50	1.07 ±0.26 1.30 ±0.48 1.50 ±0.51 1.57 ±0.51 2.28 ±0.72

Table A.18. Average R Values of Last 5 Minutes of Exercise (60% Age-predicted Maximum Heart Rate).

Subject	Group M	Group F	Group G
1	0.86 ± 0.01	0.90 ± 0.04	0.89 ± 0.04
2	0.87 ± 0.01	0.89 ± 0.02	0.94 ± 0.01
3	0.92 ± 0.02	0.88 ± 0.02	0.89 ± 0.04
4	0.90 ± 0.02	0.93 ± 0.01	0.92 ± 0.03
5	0.96 ± 0.02	0.95 ± 0.04	0.90 ± 0.03
6	0.83 ±0.01	0.86 ± 0.02	0.90 ± 0.02
7	0.89 ± 0.02	0.80 ± 0.02	0.88 ± 0.01
8	0.87 ± 0.01	1.02 ± 0.03	
9	0.82 ± 0.02	0.88 ± 0.04	
10	0.94 ± 0.03	0.85 ± 0.01	
11		0.87 ± 0.01	
12		0.83 ± 0.01	
13		0.86 ± 0.03	
14		0.87 ± 0.02	
Group	0.891 ± 0.04	0.888 ± 0.06	0.908 ± 0.03

Values are mean ± standard deviation.
No Significant Differences among group means.

Table A.19. Average Absolute and Relative Oxygen Consumption: VO_2 (L) and VO_2 (ml/kg/min) STPD during the Last 5 minutes of Exercise (60% Age-predicted Maximum Heart Rate).

Subject	Group M		Group F		Group G	
	(L)	ml/kg/min	(L)	ml/kgmin	(L)	ml/kgmin
1	0.22±0.02	3.97±0.38	0.53±0.53	9.51±0.59	0.45±0.07	5.94±0.99
2	0.29±0.06	4.70±1.04	0.45±0.07	7.48±1.16	0.48±0.04	8.89±0.79
3	0.38±0.04	5.58±0.60	0.27±0.01	4.41±0.23	0.55±0.05	9.91±0.89
4	0.29±0.02	4.59±0.39	0.33±0.03	5.72±0.66	0.53±0.04	8.60±0.76
5	0.46±0.01	9.05±0.38	0.25±0.03	3.77±0.45	0.82±0.10	11.15±1.49
6	0.57±0.04	9.24±0.73	0.41±0.05	7.65±1.02	0.41±0.03	7.58±0.64
7	0.70±0.04	11.67±0.71	0.24±0.10	3.46±1.49	0.34±0.03	4.66±0.51
8	0.29±0.03	5.07±0.51	0.31±0.09	4.15±1.21		
9	0.57±0.03	10.32±0.57	0.50±0.04	8.33±0.65		
10	0.81±0.07	12.27±1.14	0.44±0.05	8.93±1.18		
11			0.49±0.02	9.33±0.52		
12			0.47±0.05	9.15±0.95		
13			0.97±0.05	16.11±0.94		
14			0.29±0.04	5.20±0.69		
Group	0.46±0.19	7.64±3.11	0.43±0.18	7.52±3.32	0.51±0.15	8.10±2.27

Values are mean ± standard deviation.
No Significant Differences among group means.

Table A.20. Average Carbon Dioxide (VCO_2 in L/min) STPD Production in the Last 5 Minutes of Exercise (60% Age-predicted Maximum Heart Rate).

Subject	Group M	Group F	Group G
1	0.19±0.02	0.45±0.03	0.40±0.07
2	0.26±0.05	0.40±0.05	0.43±0.04
3	0.36±0.04	0.24±0.01	0.50±0.05
4	0.26±0.02	0.28±0.02	0.48±0.05
5	0.44±0.02	0.22±0.03	0.76±0.10
6	0.48±0.03	0.35±0.05	0.37±0.04
7	0.63±0.04	0.21±0.08	0.32±0.04
8	0.25±0.02	0.25±0.08	
9	0.47±0.02	0.43±0.04	
10	0.76±0.08	0.42±0.05	
11		0.46±0.02	
12		0.42±0.04	
13		0.99±0.08	
14		0.26±0.03	
Group	0.41±0.17	0.39±0.19	0.46±0.14

Values are means ± SD.
No Significant Differences among group means.

Table A.21. Average VE (BTPS) during the Last 5 minutes of Exercise (60% Age-predicted Maximum Heart Rate).

Subject	Group M	Group F	Group G
1	5.57±0.73	15.70±1.03	14.18±2.59
2	8.18±1.67	13.85±1.26	12.38±1.10
3	11.71±1.18	7.74±0.67	16.64±1.15
4	12.16±1.08	11.98±0.83	15.06±1.87
5	17.93±1.03	6.84±1.12	20.82±2.16
6	17.26±1.16	10.91±1.48	12.25±1.78
7	18.29±1.22	10.00±0.70	9.31±1.03
8	8.93±0.85	8.09±3.09	
9	15.32±0.70	14.93±1.62	
10	22.89±2.31	12.12±2.13	
11		15.67±0.87	
12		12.72±1.62	
13		23.54±2.73	
14		7.87±1.15	
Group	13.82±5.30	12.34±4.59	14.37±3.82

Values are mean ± standard deviation.
No Significant Differences among group means.

Table A.22. Average Percent Carbohydrate Used as a Fuel Substrate During the Last 5 minutes of Exercise (60% Age-predicted Maximum Heart Rate).

Subject	Group M	Group F	Group G
1	55.41±3.97	51.32±5.29	64.69±15.04
2	59.11±6.39	61.32±16.02	63.49± 4.69
3	76.08±7.10	60.46±5.58	68.81± 7.01
4	68.47±7.66	40.43±14.84	68.40±13.00
5	88.77±7.14	53.94±13.15	75.70±11.02
6	44.81±4.61	58.09±7.55	67.05±14.04
7	67.15±7.08	34.03±37.01	82.63± 5.97
8	58.44±5.74	34.01±8.83	
9	43.41±7.39	54.68±9.58	
10	82.26±9.96	84.19±11.47	
11		80.04±3.71	
12		62.45±8.43	
13		98.07±5.06	
14		67.13±8.81	
Group	64.39±15.79	63.20±16.82	70.11±12.12

Values are mean ± standard deviation.
No Significant Differences among group means.

Table A.23. Average Kilocalories Expended Per Liter of Oxygen Consumed During the Last 5 minutes of Exercise (60% Age-predicted Maximum Heart Rate).

Subject	Group M	Group F	Group M
1	4.87±0.01	4.86±0.01	4.91±0.05
2	4.89±0.02	4.90±0.05	4.90±0.01
3	4.95±0.02	4.89±0.02	4.92±0.02
4	4.92±0.02	4.82±0.05	4.92±0.04
5	5.00±0.02	4.87±0.04	4.95±0.04
6	4.84±0.01	4.88±0.02	4.92±0.05
7	4.92±0.02	4.80±0.13	4.98±0.02
8	4.89±0.02	4.80±0.03	
9	4.83±0.02	4.87±0.03	
10	4.97±0.03	4.98±0.04	
11		4.97±0.01	
12		4.90±0.03	
13		5.03±0.01	
14		4.92±0.03	
Group	4.91±0.05	4.91±0.06	4.93±0.04

Values are mean ± standard deviation.
No Significant Differences among group means.

Table A.24. Average Kilocalories Expended Per Minute During the Last 5 Minutes of Exercise (60% Age-predicted Maximum Heart Rate).

Subject	Group M	Group F	Group G
1	1.00 ±0.00	2.70 ±0.48	2.40 ±0.69
2	1.50 ±0.52	2.20 ±0.42	2.30 ±0.48
3	2.00 ±0.00	1.00 ±0.00	2.80 ±0.42
4	1.20 ±0.42	1.77 ±0.44	2.70 ±0.48
5	2.00 ±0.00	1.10 ±0.31	4.30 ±0.67
6	3.00 ±0.00	2.10 ±0.31	2.00 ±0.00
7	3.60 ±0.51	1.40 ±0.51	1.80 ±0.42
8	1.50 ±0.52	2.1 ±0.31	
9	3.00 ±0.00	2.3 ±0.48	
10	4.00 ±0.47	4.9 ±0.31	
11		1.4 ±0.51	
12			
13			
14			
Group	2.28 ± 1.05	2.11± 0.94	2.55 ± 0.76

Values are mean ± standard deviation.
No Significant Differences among group means.

Table A.25. Average Work (KPMs) during the Last 5 Minutes of Exercise (60% Age-predicted Maximum Heart Rate).

Subject	Group M	Group F	Group G
1	71.0 ±15.99	271.5 ±8.2	126.9 ±4.84
2	109.3 ±5.49	205.6 ±33.43	236.4 ±24.84
3	206.9 ±7.03	154.0 ±0.00	267.0 ±12.38
4	149.2 ±3.48	143.2 ±13.84	210.9 ±10.53
5	169.4 ±10.46	100.0 ±12.20	282.1 ±16.98
6	229.3 ±12.05	252.7 ±7.46	247.0 ±0.00
7	298.9 ±6.29	151.1 ±39.14	256.1 ±23.64
8	108.8 ±13.79	189.9 ±9.9	
9	277.0 ±0.00	255.4 ±14.13	
10	290.0 ±31.96	369.0 ±0.00	
11		92.0 ±9.60	
12			
13			
14			
Group	190.98±79.31	190.40±94.47	232.34±50.54

Values are mean ± standard deviation.
No Significant Differences among means.

Table A.26. Average Work (Watts) during the Last 5 minutes of Exercise (60% Age-predicted Maximum Heart Rate).

Subject	Group M	Group F	Group G
1	11.80	44.30	20.50
2	18.10	33.60	38.50
3	33.70	25.00	43.50
4	24.60	23.50	34.30
5	27.60	1.80	46.30
6	37.50	41.30	40.00
7	48.90	13.80	41.90
8	17.80	24.80	
9	45.00	34.00	
10	47.40	31.10	
11		32.80	
12		41.80	
13		60.00	
14		15.40	
Overall Group Average ± SD	31.24 ± 12.87	30.83± 14.27	37.85± 8.36

Values are mean ± standard deviation.
No Significant Differences among means.

APPENDIX B. BLOOD ANALYSES DATA

Table B.1. Serum Estrogen Levels of Subjects at Rest (pg/ml).

Subject	Group M	Group F	Group G
1	38.20	39.61	6.28
2	43.46	44.30	3.56
3	41.39	99.23	3.20
4	92.43	425.98	<1.00
5	50.10	91.36	3.75
6	97.70	60.92	20.71
7	69.08	553.31	12.67
8	12.25	83.67	
9	80.92	149.07	
10	38.83	117.18	
11		58.47	
12		782.77	
13		80.26	
14		456.27	
Group	56.43± 27.4[a]	217.31± 236.25	7.31± 6.98 [b]

Values are mean ± standard deviation.
[a] Different from Group F ($p < .05$).
[b] Different from Group F ($p < .01$).

Table B.2. Fasting Lipids of Subjects (mg/dl).

SUBJECT	TOTAL CHOLESTEROL			HDL CHOLESTEROL			LDL CHOLESTEROL			TRIGLYCERIDES		
	Group M	F	G	Group M	F	G	Group M	F	G	Group M	F	G
1	128	115	167	54	49	55	65.6	51.2	90.2	42	74	109
2	177	210	161	30	61	66	90	135.4	82.6	285	68	62
3	152	195	207	33	69	57	101.4	111	133.8	88	75	81
4	164	146	166	53	56	51	96.2	77.8	101.6	74	61	67
5	164	154	161	46	50	42	100.4	91.4	108.6	88	63	52
6	149	185	102	49	70	50	91.4	106.6	47	43	47	25
7	155	209		50	58		89.2	130.4		79	103	
8	166	151		78	52		78.6	89		47	50	
9	180	169		46	56		112.2	102.6		109	52	
10	150	175		44	58		93	102		65	75	
11		167			63			96.8			36	
12		163			53			100.4			47	
13		195			54			117.8			116	
14		138			42			77.4			93	
GROUP	158.5 ±15.1	169.4 ±27.6	160.6 ±33.6	48.3 ±13.0	56.5 ±7.6	53.5 ±8.0	91.8 ±12.7	99.2 ±21.9	93.9 ±29.0	92 ±71.2	68.5 ±22.9	66 ±28.1

No significant differences among groups.

Table B.3. Hematocrit at Rest (0), at 10 and 20 Minutes during Exercise.

Subject	Time	Group M	Group F	Group G
1	0	41	37	37
	10	39	40	38
	20	40	.	37
2	0	38	37	43
	10	39	37	42
	20	38	36	43
3	0	38	40	43
	10	40	40	43
	20	40	35	40
4	0	39	39	38
	10	39	40	38
	20	37	40	38
5	0	39	36	40
	10	39	38	41
	20	40	36	42
6	0	38	40	39
	10	38	43	43
	20	36	34	45
7	0	36	3	
	10	37	41	
	20	37	40	
8	0	37	38	
	10	39	38	
	20	41	38	
9	0	41	36	
	10	41	37	
	20	39	.	
10	0	40	35	
	10	40	36	
	20	40	.	
11	0		39	
	10		.	
	20		42	
12	0			
	10			
	20			
13	0			
	10			
	20			
14	0			
	10			
	20			
Group Means±SD	0	38.7±1.6	37.6±1.6	40.0±2.5 [a]
	10	39.1±1.1	39.0±2.1	40.8±2.3
	20	38.8±1.6	37.6±2.8	40.8±3.0 [b]

Values are percent whole blood [a] Different from Group F @ 0 ($p<0.05$) [b] Different from Group F @ 20 ($p<0.05$)

Table B.4. Plasma Glucose at Rest (0), at 10 and 20 Minutes during Exercise.

Subject	Time	Group M	Group F	Group G
1	0	82	88	109
	10	78	74	108
	20	75	49	108
2	0	84	88	82
	10	91	88	93
	20	88	85	90
3	0	92	84	85
	10	96	92	84
	20	95	108	87
4	0	92	.	96
	10	89	75	95
	20	94	75	93
5	0	82	80	83
	10	86	75	86
	20	77	79	82
6	0	85	83	86
	10	85	78	85
	20	83	77	83
7	0	92	89	
	10	91	93	
	20	88	92	
8	0	89	75	
	10	83	82	
	20	84	81	
9	0	89	97	
	10	79	101	
	20	77	101	
10	0	81	89	
	10	84	86	
	20	.	76	
11	0	.	84	
	10	84	.	
	20	77	79	
12	0		80	
	10		83	
	20		.	
13	0		90	
	10		91	
	20		86	
14	0		40	
	10		87	
	20		83	
Group Means ±SD	0	86.8±4.4	82.4±12.5	90.1±10.4
	10	86.0±5.3	85.0±8.1	91.8±9.1
	20	83.8±7.3	82.3±14.0	90.5±9.5

Values are mg/dl. **No Significant Differences within or among group means.**

Table B.5. Plasma NH_3 at Rest (0), at 10 and 20 Minutes during Exercise.

Subject	Time	0 Minutes	10 Minutes	20 Minutes
1	0	14	24	20
	10	16	15	26
	20	18	8	28
2	0	14	17	36
	10	21	23	33
	20	19	27	32
3	0	27	60	22
	10	19	80	27
	20	25	130	38
4	0	18	.	15
	10	21	37	25
	20	28	37	25
5	0	27	26	28
	10	18	24	53
	20	35	23	54
6	0	15	21	27
	10	24	17	32
	20	25	19	30
7	0	16	25	
	10	20	30	
	20	26	62	
8	0	16	32	
	10	25	26	
	20	22	39	
9	0	11	15	
	10	16	18	
	20	22	18	
10	0	28	19	
	10	28	21	
	20	.	24	
11	0	.	19	
	10	34	.	
	20	35	39	
12	0		31	
	10		25	
	20		.	
13	0		26	
	10		22	
	20		22	
14	0		13	
	10		33	
	20		25	
Group Means ± SD	0	18.6±6.2*	25.2±11.9	24.6±7.3
	10	22±5.4	28.5±16.6	32.6±10.4
	20	25.5±5.8	36.3±31.1	34.5±10.5

Values are µmol/L. No Significant Differences among group means. * Different from 20 ($p < .05$).

Table B.6. Plasma Lactate at Rest (0), at 10 and 20 Minutes during Exercise.

Subject	Time	Group M	Group F	Group G
1	0	0.6	1.0	1.4
	10	1.3	0.8	2.2
	20	1.3	0.5	1.5
2	0	1.3	0.7	1.1
	10	3.4	2.6	2.1
	20	2.8	3.0	1.8
3	0	1.2	1.1	1.0
	10	1.6	2.2	1.6
	20	2.1	3.4	2.0
4	0	0.8	.	0.7
	10	1.2	2	2.0
	20	1.0	2.3	2.2
5	0	1.5	1.1	1.0
	10	3.6	1.3	2.0
	20	5.0	1.0	2.0
6	0	0.6	0.7	1.1
	10	0.9	1.1	1.7
	20	0.7	1.2	2.2
7	0	1.3	1.8	
	10	1.2	3.6	
	20	1.4	2.4	
8	0	1.5	2.0	
	10	2.3	1.1	
	20	1.6	1.3	
9	0	1.0	1.3	
	10	1.1	1.8	
	20	1.0	1.7	
10	0	1.3	1.3	
	10	3.4	2.7	
	20	.	3.4	
11	0	.	1.1	
	10	1.5	.	
	20	1.3	4.6	
12	0		1.2	
	10		1.8	
	20		.	
13	0		1.2	
	10		1.5	
	20		1.9	
14	0		0.7	
	10		3.0	
	20		2.9	
Group Means±SD	0	1.1±0.3	1.1±0.3*	1.0±0.2**
	10	1.9±1.0	1.9±0.8	1.9±0.2
	20	1.8±1.2	2.2±1.1	1.9±0.2

Values are mmol/L.
*Different from 10 ($p<0.01$) and 20 ($p<0.05$). **Different from 10 and 20 ($p < 0.01$).

Table B.7. Serum Cortisol at Rest (0), at 10 and 20 Minutes during Exercise.

Subject	Time	Group M	Group F	Group G
1	0	7.96	14.79	12.33
	10	7.11	13.84	11.95
	20	6.40	.	13.85
2	0	11.92	13.51	16.50
	10	13.52	12.60	14.74
	20	11.97	12.59	14.20
3	0	6.08	12.94	18.97
	10	5.87	11.59	21.35
	20	4.58	10.35	20.40
4	0	17.14	24.77	14.14
	10	15.16	22.79	11.39
	20	12.55	20.09	11.09
5	0	12.22	27.70	12.81
	10	10.74	23.80	11.95
	20	11.52	22.72	10.34
6	0	14.23	11.88	11.31
	10	12.62	16.05	11.35
	20	9.16	11.65	10.51
7	0	20.82	4.64	
	10	20.53	8.11	
	20	17.98	.	
8	0	20.08	10.94	
	10	16.21	18.71	
	20	13.68	20.97	
9	0	15.60	24.50	
	10	20.23	27.29	
	20	19.78	19.22	
10	0	15.24	18.21	
	10	13.78	20.49	
	20	13.32	16.70	
11	0		9.56	
	10		7.81	
	20		9.71	
12	0		16.02	
	10		14.03	
	20		12.78	
13	0		24.66	
	10		21.58	
	20		21.16	
14	0		21.42	
	10		20.61	
	20		19.05	
Group Mean±SD	0	14.13±4.76	16.82±6.88	14.34±2.88
	10	13.58±4.85	17.09±5.99	13.78±3.91
	20	12.09±4.66	16.42±4.71	13.40±3.81

Values are µg/dl. No Significant Differences among group means.

APPENDIX C. 24 HOUR DIETARY ANALYSIS

Table C.1. 24 Hour Dietary Analysis

Subject	Group M				Group F				Group G			
	Total kcal CHO	% Fat	% Pro	%	Total kcal CHO	% Fat	% Pro	%	Total kcal CHO	% Fat	% Pro	%
1	2052	38	18	43	1785	19	15	66	1733	25	19	56
2	1218	28	23	49	1076	24	16	60	2060	24	13	63
3	1406	18	15	66	1453	26	25	49	1713	18	19	63
4	1879	28	16	55	1809	22	19	58	1934	29	18	53
5	3195	28	23	48	2574	27	27	46	2352	44	19	37
6	896	44	19	36	1377	35	19	45	1109	35	15	49
7	2200	41	9	50	2107	24	20	56	3018	11	11	78
8	1976	28	12	60	820	29	12	59				
9	2429	31	26	43	2439	37	16	47				
10	3052	31	15	53	3843	18	11	71				
11					1683	23	32	45				
12					1411	35	19	46				
13					1983	24	18	57				
14					3455	33	18	49				
Mean ±SD	2030.3 ±741.6	31.5 ±7.6	17.6 ±5.2	50.3 ±8.7	1979.6 ±855.6	26.8 ±6.0	19.0 ±5.6	53.8 ±8.3	1988.4 ±593.6	26.5 ±10.8	16.2 ±3.3	57.0 ±12.8

No Significant Differences among group means.

APPENDIX D. INFORMED CONSENT

COLLABORATIVE STUDY OF LSU DEPARTMENT OF KINESIOLOGY AND WOMAN'S HEALTH RESEARCH INSTITUTE: THE EFFECTS OF HYPOESTROGENISM ON RESTING AND ACUTE SUBMAXIMAL EXERCISE METABOLISM USING THE GNRHaa MODEL INFORMED CONSENT

I, _____, volunteer to take part in the following research investigation.

I understand that to be eligible for this study I must fit the research volunteer criteria, which includes: 1) 21-42 years of age; 2) non-smoker; 3) normal weight; 4) non-athletes; 5) have normal and regular menstrual periods and not be post-menopausal; 6) not taking any medication, including birth control pills or hormone replacement therapy, unless being treated by a physician with GnRHaa therapy (a medication that stops the normal menstrual cycle in premenopausal women).

PURPOSE OF THE STUDY

The purpose of this research is to study the consequences of low hormone (estrogen) levels on the body's metabolism at rest and during moderate exercise. This will be done by comparing women with different hormone (estrogen) levels. For example, the lowest level of estrogen is at the time of menses, and highest levels are found about four days before ovulation. These levels will be confirmed by blood analysis.

The goal of the study is to increase understanding of how and why many females gain weight during menopause (the change of life), a time in which a woman's body stops producing estrogen. This research will focus on the connection between the absence of estrogen, a condition which occurs normally at menopause, and the body's energy balance.

DETAILS OF THE STUDY

I understand I am being asked to participate in a study that will require me to complete a one-time 30 minute exercise test on a stationary bicycle at a moderate level of intensity (50% of maximal effort). I will be placed in one of four groups of women according to when testing occurs during my menstrual cycle. The groups are as follows:

Group 1: Ten women who currently have normal menstrual periods. They will be tested during the time they are having a period.

Group 2: Ten women who currently have normal menstrual periods. They will be tested during the early part of their 28 day cycle (between days 8-12).

Group 3: Ten women who are currently receiving a medication from their gynecologist that stops their normal menstrual cycle and have been on this medication for at least two months and are otherwise healthy.

Group 4: Ten women who have volunteered to be placed on a medication by their gynecologist to stop their normal menstrual cycle but have not started this drug therapy yet. They will be tested before they begin and while on medication.

STUDY PROCEDURES FOR DATA COLLECTION

I understand that I will be asked to perform an exercise test on a stationary bicycle at below maximal intensity, and breathe through a tube connected to an instrument that will measure the volume of air I breathe and oxygen I use, as well as the carbon dioxide I blow off during the test. The test will begin at a relatively low exercise intensity and will increase in intensity every two minutes for two or three stages. The intensity of this test will be equal to a brisk walk. An intravenous (IV) catheter (small tube) will be placed in my arm by a Registered Nurse. This will allow blood samples to be drawn prior to the test and at three times during the test (@ 10, 20, and 30 minutes). I understand I will have a small amount of IV fluid (normal saline) passed into the IV catheter during the exercise test so that blood will be easily drawn. Approximately 8 ml of whole blood (about 1½ teaspoons) will be collected per draw representing less than 3 ounces total during the entire exercise test.

Before the testing date, I will be asked to comply with the following:

- Get a good night's sleep before the day of the test
- Abstain from food and drink (other than water) from midnight until testing time
- Avoid moderate or vigorous physical activity within 12 hours of testing
- Empty my bladder prior to testing

- Abstain from alcohol consumption within 48 hours of testing
- Take no over-the-counter drug, i.e., aspirin, motrin, tylenol, cold & sinus medications, etc. within 24 hours of testing
- Wear loose clothing such as a warm-up suit and rubber soled shoes for walking or running for the test procedure.

RISKS AND DISCOMFORTS ASSOCIATED WITH TESTING PROCEDURES

Exercise: I understand the risks of "submaximal" exercise testing (exercising below maximal effort), according the American College of Sports Medicine 1995 guidelines, are extremely small for my age. However, emergency equipment and trained personnel are readily available during the exercise test.

Having blood drawn through an intravenous (IV) line: I have been told that some risks connected with having my blood drawn are

- temporary discomfort from the needle stick;
- bruising (and rarely infection) at the needle stick site;
- possible fainting after blood sampling;
- possible irritation (redness) along the vein from which blood will be drawn.

I understand that if I tend to faint when I have my blood drawn, I must report it so that safety measures can be taken to prevent this from happening. I understand I will be lying down when the IV is inserted into my arm, and it will be taped securely. After leaving the testing area, I understand I should report any development of redness or infection around the IV site immediately to the research office (231-5275) so it can be evaluated promptly.

BENEFITS

I understand that by being in this study I will be contributing to scientific knowledge concerning the metabolism (energy burned at rest and during exercise) of postmenopausal females, and to help in determining why many of these women gain weight and have difficulty losing weight.

I, personally, will gain information about my fitness level. I will be given a copy of the results of all the fitness testing which will demonstrate my current overall fitness level. This information can be used as a basis for an exercise prescription and assist me in developing my fitness goals. The "Personal Fitness Profile" that I will receive will include the results of the preliminary physical fitness testing, e.g., muscular strength and flexibility; bloodwork, e.g., blood sugar, blood lipid profile; bone thickness scan; body fat percent; lung function test; and a nutritional assessment, based on a three day dietary log that I will be asked to keep prior to the stationary bicycle test. This profile will given to me at no charge.

CONFIDENTIALITY

I have been told that my privacy as a research volunteer will be protected and my identity will not be revealed. I have also been told that the information collected during this study will not be used in any way that would subject me to public embarrassment or shame. Strict confidentiality will be maintained by the study staff. I understand that results of this study may be published, but my privacy will be protected and my name and identity will not be published. I understand that my research medical record will be kept private and will only be used by staff directly involved with this study. It may, however, be inspected by staff from the Louisiana State University Institutional Review Board, but will still remain as private information.

COMPENSATION

I understand that LSU and Woman's Health Research Institute are paying for all testing and personnel and I am not responsible for any payments associated with this study. I understand if I am being treated with medication (GnRHaa) to stop my normal menstrual cycle that I am responsible for purchasing this medication prescribed to me by my physician, since I would be taking this medication even if I chose not to participate in this study.

RESEARCH VOLUNTEER RIGHTS

I understand that I may choose not to participate in this study, and I may withdraw from the study at anytime with no penalty. This will not have any effect on any treatment, if applicable, at

the present time or in the future by my personal physician or staff of the Woman's Health Research Institute or Woman's Hospital.

I have completely read this consent form. The study has been explained to me and all my questions have been answered in person. I understand the purpose of the study and the methods that are involved and any possible risks and discomforts. If I have any questions at any time during the study, I know I can call the co-investigators, Sheri Melton at (504) 763-9167 or Dr. Ellen Brooks at (504) 231-5390, or the principal investigator, Dr. Arnold Nelson at (504) 388-2036. I understand that this study has been approved by the Louisiana State University Institutional Review Board (IRB) and the Woman's Hospital Research and Development Council. I understand if I have any questions about my rights as a research volunteer or if I wish to report any concerns or complaints regarding this study, I can contact the Vice Chancellor of the L.S.U. Office of Research and Economic Development at (504) 388-5833.

INFORMED CONSENT SIGNATURES

I have received a signed copy of this consent form.

Research Volunteer Name Printed DOB	Volunteer Social Security #	Volunteer

Research Volunteer Signature	Date/Time

Witness

Arnold Nelson, Ph.D. LSU Department of Kinesiology
Principal Investigator

Sheri Melton, Ph.D. Candidate LSU
Co-investigator

Ellen Brooks, R.N., Ph.D. Woman's Health Research Institute, Co-investigator

Hoyt Ayres, M.D. Woman's Health Research Institute, Study Medical Monitor

VITA

Sheri Anne Melton was born in Dallas, Texas, on January 12, 1947. She attended both public and parochial schools in Dallas, and graduated from Bishop Dunne Catholic High School in 1965. For two years she worked as an accounting clerk at several Dallas-based companies. She moved to New Orleans in 1967 where she attended Loyola University. She graduated from that institution in 1971 with a bachelor of arts degree in Economics.

For the next fourteen years, she held several positions in banking, urban planning, marketing and hospital unit administration. In 1985, she made the decision to enter graduate school in Health and Physical Education at the University of New Orleans, where she graduated in 1987. Her professional career after graduation encompassed personal trainer, fitness consultant, manager of a fitness center, orthopedic rehab and cardiac rehab. During these years, her athletic interests and abilities grew. She competed in biathlons, triathalons, running, cycling and won several awards in her age division. In 1990, she competed in the United States Cycling Federation National Championships, Masters Time Trial (40km). In 1992, she earned a first degree black belt in Tae Kwon Do.

In 1993, she was accepted into the doctoral program in the Kinesiology Department at Louisiana State University, where she worked as a graduate assistant teaching tennis, exercise physiology labs and several undergraduate lecture classes, including community health and exercise testing and prescription. During her years at L.S.U., she was involved in several animal research projects.

Her major research interest is women's health, especially postmenopausal issues relating to estrogen status and its relationship to metabolism. She has pursued her doctor of philosophy degree in Kinesiology with a major emphasis in Exercise Physiology and a minor in Nutrition. Upon graduation in December of 1997, she plans to pursue a teaching/research/writing career.

DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Sheri Anne Melton

Major Field: Kinesiology

Title of Dissertation: The Effects of Hypoestrogenism on Rest and Acute Submaximal Exercise Metabolism Using a GnRH Agonist Analogue Model

Approved:

Major Professor and Chairman

Dean of the Graduate School

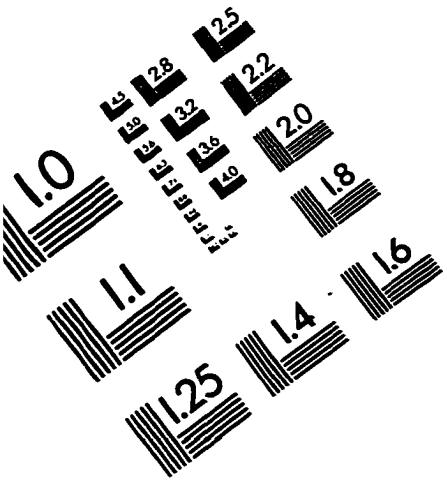
EXAMINING COMMITTEE:

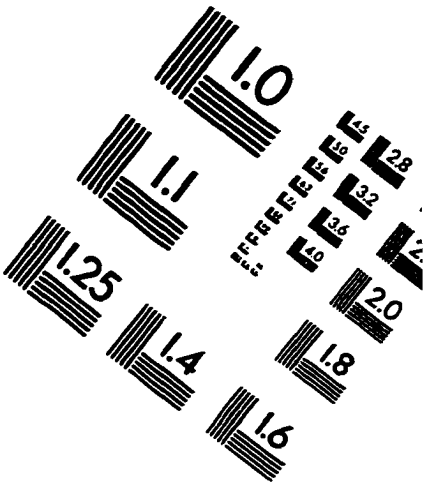
"Dissenting"

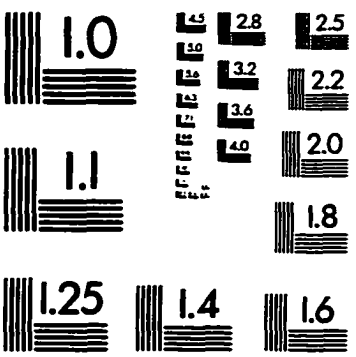
Date of Examination:

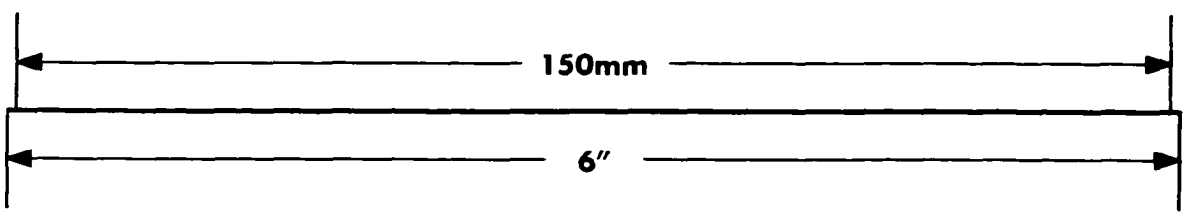
August 20, 1997

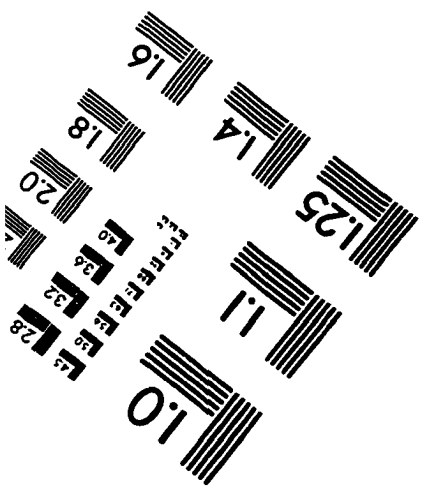
IMAGE EVALUATION TEST TARGET (QA-3)

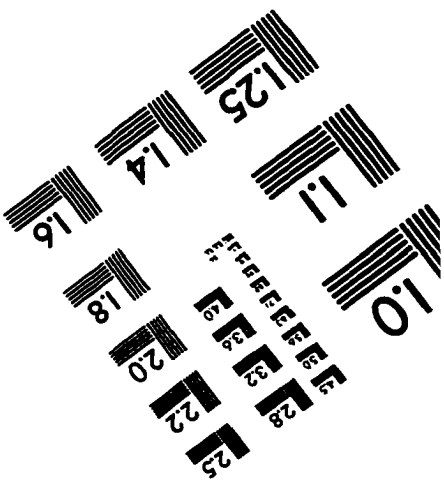












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