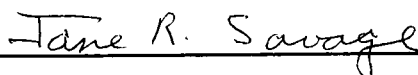


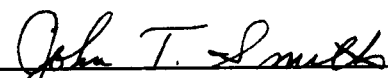
To the Graduate Council:

I am submitting herewith a dissertation written by Donna Snyder Pierce entitled "The Effect of Weight Loss on Serum Androgens in Morbidly Obese Females." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Human Ecology.


Roy E. Beauchene, Major Professor

We have read this dissertation
and recommend its acceptance:







Accepted for the Council:


Vice Provost
and Dean of the Graduate School

THE EFFECT OF WEIGHT LOSS ON SERUM ANDROGENS
IN MORBIDLY OBESE FEMALES

A Dissertation
Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Donna Snyder Pierce

June 1987

Dedication

I dedicate this work to my mother,
Barbara Rose Snyder

ACKNOWLEDGMENTS

The completion of this manuscript would not have been possible without the relentless effort of several people. I wish to thank:

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ABSTRACT

Changes in serum dehydroepiandrosterone sulfate (DS), free testosterone (FT), estrogen (E), and sex hormone binding globulin (SHBG) were studied in 50 morbidly obese women (38 premenopausal, 12 postmenopausal) who participated in a 6 month weight reduction program.

The natural log of the initial hormone levels were regressed with body weight, percent of ideal body weight, percent body fat, body mass index and weight and height in premenopausal obese, postmenopausal obese and lean premenopausal controls. Analysis of changes in hormone levels after 3 and 6 months participation in the weight loss program were determined on pre- and postmenopausal obese women.

The postmenopausal obese women had significantly lower initial DS and E levels than did the premenopausal groups of women.

The premenopausal obese women (109 kg body weight) demonstrated a significant direct relationship between initial LFT (log FT) and body weight adjusted for age as well as LFT and percent ideal body weight adjusted for age, LFT and percent body fat adjusted for age and LFT and body mass index adjusted for age.

Postmenopausal obese women (92 kg body weight) demonstrated a significant direct relationship between initial LE (log LE) and body weight, LE and percent of ideal body weight, LE and percent body fat, LE and weight and height, and LE and body mass index.

After 3 months into the weight reduction program, 19 premenopausal obese women lost an average of 4.4 kg and 7 postmenopausal obese women gained an average .4 kg. There were 8 premenopausal obese women in the study for 6 months, and they lost an average of 12.8 kg. The premenopausal obese women showed significant reductions in FT after 6 months weight reduction. There were no significant changes in E levels in postmenopausal obese women.

This study supports the theory that weight loss tends to normalize altered FT and SHBG levels in obese premenopausal women. The lack of obese postmenopausal women participation throughout the 6 month weight reduction phase limited conclusive findings for this sub-group of obese women. Exercise appeared to aid the weight loss process and the normalization of hormones in this study.

TABLE OF CONTENTS

CHAPTER	PAGE
I. INTRODUCTION.	1
II. LITERATURE REVIEW	3
III. PROCEDURES.	10
Subjects.	10
Methodology	10
Hormone Assays.	12
Dehydroepiandrosterone Sulfate	12
Reagents.	12
Procedure	13
Free Testosterone	13
Reagents.	14
Procedure	14
Total Estrogen.	14
Reagents.	15
Procedures.	15
Sex Hormone Binding Globulin.	16
Reagents.	17
Procedures.	17
Statistical Analysis.	18
IV. RESULTS	20
Initial Values.	20
Relationships Among Initial Values.	22
Effect of Weight Loss on Serum Hormone.	31
Effect of Exercise on Serum Hormones.	38
V. DISCUSSION.	41
Suggestions for Future Research	45
Summary	46
LITERATURE CITED.	49
APPENDIXES.	57
Appendix A.	58
Appendix B.	61
VITA.	63

LIST OF TABLES

TABLE	PAGE
1. Initial Body Measurements and Initial Serum Hormone Levels of Premenopausal Obese (PREO), Postmenopausal Obese (POSTO) and Premenopausal Lean (PREL) Women	21
2. Regression Equations Describing the Relationships Between the Natural Log of Serum Levels of Free Testosterone (LFT), Age and Body Measurements in Premenopausal Obese Women (PREO, n = 38).	23
3. Regression Equations Describing the Relationships Between the Natural Log of Serum Levels of Total Estrogen (LE), Body Measurements in Postmenopausal Obese Women (POSTO, n = 12)	26
4. Regression Equations Describing the Relationships Between the Natural Log of Serum Levels of Total Estrogen (LE), Body Measurements and Age in Postmenopausal Obese Women (POSTO, n = 12)	28
5. Initial Values and Changes in Body Weight of Premenopausal Obese (PREO) and Postmenopausal Obese (POSTO) Women Who Participated for 3 or 6 Months in the Weight Reduction Program	33
6. Initial Values and Changes in Serum Hormone Levels of Premenopausal Obese (PREO) Women Who Participated for 3 or 6 Months in the Weight Reduction Program	35
7. Initial Values and Changes in Serum Hormone Levels of Postmenopausal Obese (POSTO) Women Who Participated for 3 Months in the Weight Reduction Program.	36
8. Initial Values and Changes in Serum Hormone Levels in Premenopausal Obese (PREO) Women Who Lost at Least 10 kg After Participating 3 or 6 Months in the Weight Reduction Program	37
9. Comparison of Serum Hormone Levels of Exercising and Non-Exercising Premenopausal Obese (PREO) Women Prior to Participation in the Weight Reduction Program.	39

TABLE

PAGE

10. Initial Values and Changes in Serum Hormone Levels of Exercising Premenopausal Obese (PREO) Women Who Participated for 6 Months in the Weight Reduction Program	40
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LIST OF FIGURES

FIGURE	PAGE
1. Relationship of natural log of free testosterone (LFT) to weight adjusted for age (years) in premenopausal obese women (PREO).	25
2. Relationship of natural log of estrogen (LE) to body mass index in postmenopausal obese women (POSTO)	27
3. Relationship of natural log of estrogen (LE) to age in postmenopausal obese women (POSTO).	29
4. Relationship of natural log of estrogen (LE) to body mass index adjusted for age (years) in postmenopausal obese women (POSTO)	30
5. Relationship of natural log of sex hormone binding globulin (LSHBG) to weight in premenopausal lean women (PREL). . . .	32

CHAPTER I

INTRODUCTION

The relationship between obesity and adrenocortical function has been the subject of several studies (1-7). While alterations in serum adrenal hormone levels are accepted features of obesity (3, 7-10), it is not clear whether they are primary effectors or secondary results.

Hyperandrogenism in women has not only been associated with hirsutism (11-13) but with infertility (11) and obesity (10, 13, 14) as well. Changes in serum levels of androgens have been associated with obesity (13-27) and with weight loss (28, 30-33). Often serum total testosterone levels have been measured in obesity studies and frequently were found to be within normal ranges (15, 17, 18, 22). However, decreased levels of sex hormone binding globulin (SHBG), a common finding in the obese (20-22, 34, 67), may be related to an elevation in the free testosterone portion of the blood in obese subjects (21, 22, 67).

Serum levels of free testosterone and dehydroepiandrosterone sulfate have been found to be frequently elevated in women suspected of having hyperandrogenism (14, 17, 67). Free testosterone is a bioactive steroid and may be more indicative of androgenicity than total testosterone alone. Since elevations of bioactive steroids, e.g., androstenedione, free testosterone and dehydrotestosterone,

have been associated with weight gain in animals (1), the chronic effect of increased levels of free steroids in humans might be important in some metabolic disorders of obesity.

While there are reports of normal estrogen levels in premenopausal obese women (35-37, 39), total estrogen levels are frequently elevated in post menopausal obese women (24, 38-42). Increases in estrogen levels may be partially explained by the enhanced ability of obese women to form estrogen from circulating androstenedione (via testosterone) in adipose tissue (24, 39, 42-44).

Since much of the research investigating the relationship between obesity and hyperandrogenism has been done on males, the present study was undertaken utilizing females. Changes in dehydroepiandrosterone sulfate, free testosterone, estrogen and sex hormone binding globulin in morbidly obese women before, during and after a weight reduction program were measured. The primary objective of the study was to measure serum androgen levels in obese women and to note changes in their levels during weight loss. If elevated serum androgens were observed in obese subjects and decrements in their levels were not corrected with weight loss, the elevated steroids may be partially responsible for the obesity, i.e., predisposing those individuals to weight gain. If androgen levels decrease with weight loss, it would seem likely that the state of obesity caused the elevation of serum androgens.

Another objective of the study was to examine the effect of exercise activity on dehydroepiandrosterone sulfate, free testosterone, estrogen and sex hormone binding globulin in the women.

CHAPTER II

LITERATURE REVIEW

Androgen excess in women can arise from the ovaries and/or the adrenals. Dehydroepiandrosterone sulfate (DS), a marker of androgen activity is frequently, but not always, elevated in hirsute women (45). Lobo, et al. (11) found that in comparison with infertile and oligomenorrheic women, hirsute women more often had elevated serum DS levels. Sixty percent of hirsute women had elevated DS and 82% had either elevated DS or FT. Wild et al. (17) reported that of 138 women suspected of having hyperandrogenism, 59% had elevated DS and 92% had either elevated DS or FT.

The concentration of adrenal androgens dehydroepiandrosterone (D) and DS in the blood decline with age especially around the time of menopause (46-48). DS levels also vary with weight status (17, 29). A study by Halmy and Feher (19) reported that there was an increase in metabolic clearance rates of DS in obese women compared to normal controls. D was found to prevent obesity in one strain of mice (49, 50).

Total serum testosterone (T) has been reported to be increased and androstenedione (A) and sex hormone binding globulin (SHBG) decreased in massively obese women (14). Levels of these hormones were shifted towards normal values upon weight reduction and more normal menstrual cycles were established (28). Conflicting results

were found in a similar study done on Pima Indians by Purifoy et al. (29). They found obese Pima females had decreased SHBG and T concentrations and that age had a strong effect on androgen levels.

Free or unbound testosterone (FT) is believed to be indicative of the active androgen fraction in the blood (12, 15, 29). Vermeulen et al. (12) reported that, although variable, the plasma FT levels were more frequently elevated than T in hirsute women. Elevated FT levels may be related to the decrease in SHBG that has been observed in hirsute and obese women (29). Vermeulen et al. (34) studied factors that affect SHBG levels in the blood. Before puberty SHBG levels were similar in both sexes but in adulthood, women had higher levels ($7.4 \pm 0.8 \times 10^{-8}$ moles/l) than did men ($4.6 \pm 0.4 \times 10^{-8}$ moles/l). Elevated levels were found in men with hypogonadism and in hyperthyroidism. Pregnancy increased SHBG levels but after delivery decrements were observed. Decreased SHBG values were found in hirsutism, nephrotic syndrome and obese women. Not only have hirsute and obese women been found to have decreased levels of SHBG but in women who are both hirsute and obese, a further lowering of SHBG has been observed (20).

Normal levels of T have been observed in hirsute women (12, 15) and researchers have suggested that measurement of FT and SHBG would be more indicative of androgen activity (15, 16). Haymond et al. (15) in a case study of an 11-year-old premenarchial girl who had clinical symptoms of Stein-Leventhal syndrome, reported normal levels of T but an elevated FT and depressed SHBG levels.

Low SHBG and high FT observed in obese women may be responsible for polycystic ovarian syndrome (PCO) by inhibiting follicular maturation (21, 22, 23). Depressed SHBG and increased FT have been found in obese PCO patients with normal serum T levels. Normal weight PCO patients showed similar results but the SHBG reduction was not as great and normal T levels were seen less often (22).

There also seems to be an alteration in the metabolic clearance rates of androgens in the obese. Production rates of bioactive steroids (T, D and A) in obese, hirsute, normally ovulating women were reported to be double that of normal controls (51). The increase in metabolic clearance rate of these androgens may result in decreased blood levels in the obese (52).

In vitro, the conversion of androgens to estrogens by aromatase enzymes in adipose tissue has been demonstrated. Increased extraglandular aromatization of plasma A to estrone has been observed in obese postmenopausal women (24). Edman and MacDonald (25) studied extraglandular aromatization of A in anovulatory young women. They observed greater conversion of A to estrone in the obese. When body weight is in excess of 150% ideal body weight, there is a striking difference in aromatization of plasma A. The difference between ovulatory and anovulatory obese women was not found to be significant in this study.

In a recent review article, Whitworth and Meeks (43) reported there was a relationship between body weight and aromatization of A to estrogen (E) demonstrated on the basis of multiple-body-weight-related parameters including total body weight, pounds of excess

weight, percentage of ideal body weight and body mass ratio index (WT/HT^2). This aromatization was reported to be 2 to 5 times greater in young obese women and 2 to 4 times greater in postmenopausal obese women than in lean controls (43).

Ellison (54) conducted a study with 24 young women of normal weight and reported no relationship between body weight and DS or E. To explain this observation, he suggested a set point theory in which normal E levels would exist with slight variations in body weight. In normal weight post menopausal women, Reed et al. (55) reported that there was no significant relationship between E levels and age or body weight.

Often studies on obese premenopausal women report normal levels of E in serum or urine (35-37). Zumoff et al. (35) reported normal 24-hour mean plasma concentrations of estrone or estradiol in obese cyclic women. Although there is believed to be an increase in E production in the obese due to an increase in the aromatization of A by adipose cells (42), Zumoff et al. (35) suggested that the reason plasma E levels were not significantly elevated in obese premenopausal women was that the small amount derived from A was swamped by the much larger amount derived from ovarian secretion which was apparently unaffected by obesity (35). Loughlin et al. (44) studied premenopausal amenorrheic obese women. They reported elevated serum levels of estrone but normal estradiol levels.

Cleland et al. (42) studied the effects of aging as well as body weight on aromatase activity of adipose tissue. They reported

a significant increase in the aromatase activity in adipose cells from postmenopausal compared to premenopausal women. There was a significant correlation reported between E formation and body weight and a 2 to 4 fold increase in E production was associated with aging. They concluded that the increase in E formation associated with aging was probably a result of increased adipose tissue aromatase activity per adipose cell. On the other hand, the increase in E formation in the obese was probably due to an increase in adipose cell number (42).

Jensen et al. (41) found no relationship between age and E levels in two groups of postmenopausal women (one group 46-56 years old, another group 70 years and older). They reported an increase in fat mass in the older group with a significant correlation between fat mass and serum E (41).

Friedman and Kim (39) suggested that in postmenopausal women the increased estrogenic state associated with obesity can be clearly demonstrated by hormonal measurement, as well as increased risk of endometrial cancer and a decreased risk of osteoporosis. In ovulatory women, the increased E levels have not been as clearly documented perhaps due to the fact that ovarian production minimizes the peripheral contribution, preventing its detection in cross sectional studies (39).

Weight loss also exerts an effect on hormonal status. In postmenopausal women weight loss was associated with an increase of SHBG to a level higher than that of nonobese postmenopausal women (30). Friedman et al. (31) found a reduction in FT levels and an

increase in SHBG after weight loss in premenopausal obese women. It was reported also that high density lipoprotein cholesterol increased and low density lipoprotein cholesterol decreased after weight reduction was achieved.

de Waard et al. (38) measured urinary excretion of estrone and estradiol in 42 obese postmenopausal women before and after 6 and 12 months participation in a weight reduction program. They reported a significant correlation between E and the Quetelet body mass index (WT/HT^2) before weight reduction effort. An average weight loss of 4.5 kg and a normalization of E levels was reported at the end of the 12-month weight loss period (38).

Normalization of A and E production has been reported with weight loss in formerly obese patients. Metabolic abnormalities such as increased aromatization of A to E and accelerated metabolic clearance rates of A remain abnormal after weight loss (56). In a study by Harlass et al. (57), 6 amenorrheic and/or infertile women on a weight loss program experienced decreases in serum levels of both T and FT. Stanik et al. (33) studied changes in hormone levels after weight loss in moderately obese men. They found that pre-weight loss measurements indicating elevated FT, decreased SHBG and normal T was in keeping with data on heavier men (26, 27). After the weight loss, hormone levels returned to normal.

Androgens may be associated with hirsutism, infertility, hyperinsulinism, as well as obesity. Levels of these hormones can be influenced by a variety of factors such as age, sex, diet, pregnancy,

ethnic background and disease adding to the complexity of this problem. Thus, the role androgens play in obesity is unclear but the evidence suggests relationships that warrant further investigation.

In summary the literature indicates that in women hyperandrogenism can result from an over production of androgens from the adrenal glands or the ovaries. Elevated E can also result from excessive conversion of A to E in fat tissue. An increase in metabolic clearance rates of hormones in the obese can cause serum levels to appear low or normal when androgen production is high.

DS levels have sometimes been found to be elevated in the obese women, but not always. Increasing age will usually result in decreased DS levels in the blood, particularly after menopause.

T is often found to be normal in obese women. It can be elevated. More often FT is elevated probably because of a decrease in SHBG, a common finding in the obese. E has not been found to be altered in the obese until recently. Elevated E has been seen more often in the postmenopausal obese probably due to increase aromatase activity in fat cells. In premenopausal obese women it is difficult to detect excess conversion of A to E via aromatization due to large ovarian secretion of E.

Recent studies have shown a trend towards a normalization of serum hormone levels accompanying weight loss in the obese; however, preliminary studies of metabolic alterations in aromatase activity and hormone clearance rates in the obese have shown that these alterations may remain abnormal after weight loss.

CHAPTER III

PROCEDURES

Subjects

Obese participants were recruited from the Weight Control Unit at Columbia University Obesity Research Center, St. Lukes-Roosevelt Hospital, NY. These women were divided into two groups: premenopausal obese women (PREO), n=12 and postmenopausal obese women (POSTO), n=38. Other subjects were premenopausal lean women (PREL), n=29 who were participating in a larger study designed to investigate the relationships between lipids and androgens at the University of Tennessee Memorial Hospital and Research Center, Knoxville, Tennessee (58). (Women on birth control pills or other hormone medication were not allowed to participate in the study.)

Methodology

A physical exam was administered to all participants and evaluated for acceptance into the study. On the day of the physical examination, the patient was advised of potential risks and asked to participate in the study and to sign a consent form (Appendix A). The subject's age, name, phone number, present height and weight were recorded. The date of the first day of her last menstrual period (LMP) was recorded. A blood sample (10 ml) was collected from each

subject during the 5-14th days of her menstrual cycle. Blood samples were centrifuged and the serum separated from cells. The serum was frozen and stored at -20°C .

Percent of ideal body weight was calculated for all subjects using the Fogerty Method of estimation based on the 1959 Metropolitan Life Insurance Company table (59). In addition to the above, the obese women (PREO and POSTO) participated in weekly meetings consisting of group discussions and problem solving with emphasis on behavior modification techniques. Their weight reduction diet was based on caloric need determined by indirect calorimetry and food records. A deficit of between 500 and 1000 kilocalories per day was recommended for a 1 to 2 pound weight loss per week.

Obese women informed the investigator when their menstrual cycle began and there was a blood sample collected once a month for six months. Serum was frozen and stored at -20°C . At 3 month intervals the serum samples of the obese women were shipped United Parcel Service on dry ice to the Tennessee Endocrine Reference Laboratory in Knoxville, Tennessee. The hormone assays were conducted in Knoxville by the investigator. When the monthly blood samples were collected from the obese women, weight, exercise and menstrual cycle information were recorded again (Appendix B).

Percent body fat was estimated by total body potassium in the obese women (60). To investigate the effect of exercise on serum androgen levels premenopausal obese women were divided into exercisers and non-exercisers. Only organized exercise classes or work-outs,

e.g., swimming 3 days a week for 30 minutes, were considered adequate for placement in the exercise group.

Hormone Assays

Dehydroepiandrosterone Sulfate

The Radioassay Systems Laboratory (Palo Alto, CA) radio-immunoassay method (61) for analysis of dehydroepiandrosterone sulfate (DS) permits the direct quantitation of DS in serum. The method is based on a specific immunological reaction in which the "tracer" antigen (DS) is labeled with ^{125}I and is made to compete with the cold antigen for a specific limited number of binding sites on the antibody. The free or unbound tracer antigen is separated from the bound by a specific second antibody, after centrifugation. The radioactivity in the pellet is measured and a standard curve is generated using known amounts of unlabeled DS. The concentrations of unknown samples were determined from the standard curve (61). Normal female serum values for DS range from 60-230 $\mu\text{g/dl}$.

Reagents. 1. DS-3-monohemisuccinate-HSA was the antigen used to generate antiserum in rabbits. This first antibody (gamma globulin specific for DS) was dissolved in 0.01M phosphosaline buffer, pH 7.0.

2. DS standards. Seven standards were provided ranging from 0.05 mg/dl to 0.5 ng/dl dissolved in 0.01M phosphosaline buffer, pH 7.0.

3. Second antibody. Goat anti-rabbit gamma globulin dissolved in 0.01M phosphosaline buffer, pH 7.0.

4. 30% polyethylene glycol (PEG 6,000) dissolved in 0.01M phosphosaline buffer, pH 7.0.

5. ^{125}I labeled DS. This radioactive material contains less than 3 μCi per vial and was dissolved in phosphosaline buffer, pH 7.0.

Procedure. Label 10 ml conical bottom glass tubes in duplicate as follows: total count, buffer, 7 standard, quality control high and low and serum while serum samples thaw in water bath (37°C). Add 25 μl of standards or patient serum to appropriate tubes. Add 150 μl phosphosaline buffer into buffer tubes. Add 125 μl of the first antibody into all except total count and buffer tubes. Add 25 μl of ^{125}I labeled DS into all tubes. Mix. Incubate for 40 minutes at 37°C . Mix the second antibody with an equal amount of PEG. Add 50 μl of the mixture to all except total count and buffer tubes. Mix. Chill 4°C for 10 minutes. Mix. Centrifuge (Sorvall RC38, HC-46 rotor, Norwalk, CT) at 5,000 x g for 15 minutes. Aspirate the fluid in the tubes and count pellet radioactivity for 5 minutes in gamma counter (Kineticount KC-48, Medical and Scientific Designs, Rockland, MS). Serum DS values were determined using Medical and Scientific Designs software on a Hewlett Packard (#9121) computer.

Free Testosterone

Free testosterone (FT) was measured using the Diagnostic Products Corporations (Los Angeles, CA) coat-a-count procedure (62).

^{125}I labeled testosterone analog competes for a fixed time with free testosterone in the serum sample for sites on the testosterone

specific antibody adhering to the wall of a polypropylene tube. The fluid in the tube is then decanted, to isolate the antibody bound fraction and radioactivity counted in a gamma counter. The counts are inversely related to the amount of free testosterone in the sample. Normal female serum values range from 0.07-0.36 ng/dl.

Reagents. 1. Free testosterone antibody coated polypropylene tubes.

2. Free ^{125}I labeled testosterone tracer.

3. Free testosterone calibrators. Six vials of processed human serum containing 0, 0.15, 0.50, 1.50, 3.25 and 5.00 pg/dl of free testosterone.

Procedure. Label coated polypropylene tubes as follows: total count, 6 standards, quality control high and low and serum samples in duplicate while serum thaws in water bath (37°C). Add 50 μl of standard or serum to appropriate tubes. Add 0.9 ml of free ^{125}I labeled testosterone to every tube and mix. Incubate for 4 hours at 37°C. Decant fluid in the tubes thoroughly. Measure radioactivity in a gamma counter (Kineticount KC-48, Medical and Scientific Designs, Rockland, MS). Serum free testosterone values were determined using Medical and Scientific Designs software on a Hewlett Packard (#9121) computer.

Total Estrogen

The Radioassay System Laboratory (Palo Alto, CA) total estrogen (E) radioimmunoassay detects the additive concentration of unconjugated

estradiol-17 β and estrone (63). This is a double antibody radioimmunoassay. The first antibody (rabbit gamma globulin) is specific for estrone and estradiol. The second antibody (goat gamma globulin) is specific for the first antibody. This forms a large molecule that precipitates and forms a pellet. The radioactivity in the pellet can then be measured in a gamma counter. The first antibody does not react with estrogen sulfates or glucuronides. It does not distinguish between free estradiol, estrone, or estrone bound to circulating binding proteins. Thus total estrogen (E) assay is an approximation of plasma estrogenicity since estradiol is biologically more active than estrone (63). Normal female serum values range from 60-440 pg/ml.

Reagents. 1. Anti-Total Estrogens. Estradiol-17 β 3-carboxymethylether-BSA was used as the antigen to generate antiserum in rabbits. The antiserum was dissolved in 0.01M phosphosaline buffer, pH 7.0.

2. Total estrogen standards. Six standards were provided ranging from 25 pg/ml to 1000 pg/ml dissolved in 0.01M phosphosaline buffer, pH 7.0.

3. Second antibody. Goat anti-rabbit gamma globulins dissolved in 0.01M phosphosaline buffer, pH 7.0.

4. ^{125}I labeled estradiol-17 β . This radioactive material contains less than 5 μCi per vial dissolved in 0.01M phosphosaline buffer, pH 7.0.

Procedures. Label 10 ml conical bottom glass tubes in duplicate as follows: total count, buffer, 6 standards, quality control high and low and serum while serum samples thaw in water bath (37°C). Add 15 µl of standards or patient serum into appropriate tubes. Add 150 µl of phosphosaline buffer in buffer tubes. Add 25 µl of the first antibody to all but the total count and buffer tubes. Mix. Add 25 µl of ^{125}I labeled estradiol-17 β to all tubes. Mix. Incubate at 37°C for 90 minutes. Mix. Add 25 µl of the second antibody to all tubes. Mix. Chill 4°C for 10 minutes. Mix. Centrifuge (Sorvall RC3B, HC-46 rotor, Norwalk, CT) at 5,000 x g for 15 minutes. Aspirate fluid and measure radioactivity of pellet in gamma counter (Kineti-count KC48, Medical and Scientific Designs, Rockland, MS) for 5 minutes. Total serum estrogen values were determined using Medical and Scientific Designs software on a Hewlett Packard (#9121) computer.

Sex Hormone Binding Globulin

Sex Hormone Binding Globulin (SHBG) is measured via SHBG-IRMA test procedure from Farnos Laboratory via Nuclear Diagnostics, Inc. (Troy, MI) (64). The method is based on the principles of non-competitive "liquid phase" immunoradiometric assay. The radioactive isotope is bound to monoclonal SHBG antibody instead of the hormone. A second antibody (donkey gamma globulin) is specific for the first antibody forming a large molecule that precipitates and forms a pellet. Normal female serum values range from 30-90 nmol/l.

Reagents. 1. SHBG antiserum reagent. SHBG antiserum is dissolved in phosphate-buffered saline (PBS): 25.0 mmol/l phosphate, 0.9% NaCl, 0.625% lactose, 1.0% fetal bovine serum, 0.05% sodium oxide, pH 7.6.

2. SHBG standards. Six standards are provided ranging from 6.25-200.0 nmol/l from serum of pregnant humans.

3. Separation reagent. The reagent contains the second antibody (donkey anti-rabbit Ig G) covalently bound to solid particles 100 ml of the PBS buffer described above.

Procedures. Label 10 ml conical plastic tubes in duplicate as follows: total count, buffer, 6 standards, quality control high and low and patient serum while serum samples thaw in water bath (37°C). Add 20 µl of buffer, standards or patient serum into appropriate tubes. Mix together equal amounts of antiserum and ^{125}I labeled SHBG antibody and add 0.40 µl of the above mixture to each tube. Mix. Incubate at 25°C for 60 minutes. Add 100 µl of separation reagent to each tube. Mix. Add 400 µl of physiological saline (0.9 g/l) and centrifuge (Sorvall RC3B, HC-46 rotor, Norwalk, CT) at 5,000 x g for 15 minutes. Aspirate the fluid and measure radioactivity of pellet in scintillation counter (Beckman Scintillation Count LS1801, Smith-Kline-Beckman, Norcross, GA) for 5 minutes. Serum SHBG values were determined using Medical and Scientific Designs software on a Hewlett Package (#9121) computer.

Statistical Analysis

The research questions were: (1) What relationships exist among serum androgen levels and body weight parameters in women? (2) Do altered serum androgen levels in obese women tend to approach non-obese levels with weight loss? (3) Are serum androgen levels related to the amount of exercise? To investigate research question number 1, initial serum androgen levels of each of the three groups were regressed (65) with initial body measurements: body weight, percent of ideal body weight, percent body fat, height and weight, and Quetelet body mass index. Also initial mean values of each of the three groups were compared using Duncan's multiple range test (66).

To investigate research question number 2, initial and final serum androgen levels of the two obese groups were compared using a paired t-test (66).

To investigate research question number 3, initial serum androgen levels of exercising obese premenopausal women were compared to non-exercising obese premenopausal women using a paired t-test. Also initial and final serum androgen levels of the exercising obese premenopausal women were compared using a paired t-test (66).

Plots of residuals from preliminary models ($DS=AGE$, $DS=WT$, $E=AGE$, $E=WT$, $FT=AGE$, $FT=WT$, $SHBG=AGE$ and $SHBG=WT$) indicated apparent heteroscedasticity. The variance appeared to be related to the expected values. Thus the data were transformed via natural logarithm for variables DS , E , FT and $SHBG$ and the resulting pattern of errors

appeared to meet the assumed underlining analysis of variance. This transformation has been employed previously with measurement of hormones (37).

CHAPTER IV

RESULTS

Initial Values

Table 1 presents initial descriptive data for the subgroups: premenopausal obese (PREO), postmenopausal obese (POSTO), and premenopausal lean (PREL) women.

Seventy-nine subjects participated in the study. Of the 50 obese subjects studied, 38 were premenopausal (PREO) and 12 were postmenopausal (POSTO). There were 29 premenopausal lean (PREL) women.

There were significant differences in age among the PREO, POSTO and PREL. The mean age of the PREO group was 38.4 years of age and the POSTO group 58.1 years. The difference between the two was significant. The mean age of the PREL group was 31.6 years which was significantly less than that of PREO or POSTO groups. There were also significant differences in body weight among the PREO, POSTO and PREL groups. The PREO group weighed an average of 109 kg, the POSTO group 92 kg and the PREL group 60 kg. The POSTO group was significantly shorter than the PREO and PREL groups. Both the PREO and the PREL women averaged 164 cm in height and the POSTO women averaged 158 cm. The average percents of ideal body weight for the PREO and POSTO groups were 191 and 175, respectively. The differences

Table 1. Initial Body Measurements and Initial Serum Hormone Levels of Premenopausal Obese (PREO), Postmenopausal Obese (POSTO) and Premenopausal Lean (PREL) Women.

Parameter	PREO	POSTO	PREL
AGE (years)	38.4 ± 1.4 ^a (38) ^{1,2}	58.1 ± 2.1 ^b (12)	31.6 ± 1.2 ^c (29)
WT (kg)	109.4 ± 4.7 ^a (38)	92.1 ± 5.4 ^b (12)	60.0 ± 1.9 ^c (29)
HT (cm)	164.7 ± 1.0 ^a (38)	158.2 ± 2.7 ^b (12)	164.0 ± 1.3 ^a (29)
BODY WT (%) ³	190.9 ± 7.1 ^a (38)	175.1 ± 10.2 ^a (12)	100.9 ± 2.5 ^b (29)
BODY FAT (%)	55.14 ± 1.20 ^a (35)	55.25 ± 7.39 ^a (12)	- - - - -
WT/HT ² (kg/m ²)	39.8 ± 10.2 ^a (38)	36.5 ± 9.2 ^a (12)	21.6 ± 3.5 ^b (29)
DS (µg/dl) ⁴	121.8 ± 9.6 ^a (37)	86.8 ± 12.3 ^b (11)	136.3 ± 12.6 ^a (29)
FT (ng/dl) ⁵	0.27 ± 0.02 ^a (37)	0.25 ± 0.03 ^a (11)	0.20 ± 0.02 ^a (8)
E (pg/ml) ⁶	215.3 ± 19.6 ^a (32)	83.1 ± 18.9 ^b (10)	195.3 ± 11.0 ^a (26)
SHBG (nmol/l) ⁷	39.59 ± 3.12 ^a (31)	40.25 ± 4.85 ^a (12)	47.25 ± 3.92 ^a (24)

¹Values are means ± SEM for number of subjects shown in parentheses.

²Means in a row sharing the same superscript are not significant differently from each other (P>.05) using Duncan's multiple-range test (67).

³BODY WT (%) = percent ideal body weight (48).

⁴DS = serum dehydroepiandrosterone sulfate.

⁵FT = serum free testosterone.

⁶E = total serum estrogen.

⁷SHBG = serum sex hormone binding globulin.

between the average percent of ideal body weight of the PREO and POSTO groups were not significant. The PREL group had significantly lower percent of ideal body weight (101) than did both the PREO and the POSTO groups. The average body mass index for the PREO group was 39.8 kg/m^2 and for the POSTO group it was 36.5 kg/m^2 . There was not a significant difference in the average body mass index between the PREO and POSTO groups. The average body mass index of the PREL group (21.6 kg/m^2) was significantly lower than the PREO and POSTO groups. The POSTO group had significantly lower serum DS levels ($86.8 \text{ } \mu\text{g/dl}$) than the PREO ($121.8 \text{ } \mu\text{g/dl}$) and the PREL ($136.3 \text{ } \mu\text{g/dl}$) group. The POSTO group also had significantly lower serum E levels (83.1 pg/ml) than did PREO (215.3 pg/ml) and PREL (195.3 pg/ml) groups. There were no significant differences in the serum DS or E levels between the PREO and PREL groups. The serum levels of FT among the PREO, POSTO and PREL groups were not found to be significantly different from each other. No significant differences were observed in serum levels of SHBG among the 3 groups as well.

Relationships Among Initial Values

Table 2 describes the relationships between the natural log of serum levels of free testosterone (LFT) and body measurements in the PREO group. There was a low but significant direct relationship between LFT and age ($R^2=0.14$, $P<0.01$). There were also significant direct relationships between LFT, body weight, and age ($R^2=0.20$, $P<0.01$); LFT, percent of ideal body weight and age ($R^2=0.21$, $P<0.01$); LFT, percent body fat and age ($R^2=0.20$, $P<0.01$); and LFT, body mass

Table 2. Regression Equations Describing the Relationships Between the Natural Log of Serum Levels of Free Testosterone (LFT), Age and Body Measurements in Premenopausal Obese Women (PREO, n = 38).

EQUATION	R ²	P
LFT (ng/dl) = - 0.65 + 0.01 AGE (years)	0.14	0.01
LFT (ng/dl) = - 0.94 + 0.003 BODY WT (kg) - .02 AGE (years)	0.20	0.01
LFT (ng/dl) = -1.01 + 0.002 BODY WT (%) ¹ - .02 AGE (years)	0.21	0.01
LFT (ng/dl) = -1.12 + 0.01 BODY FAT (%) - .02 AGE (years)	0.20	0.01
LFT (ng/dl) = - 0.94 + 100.42 WT/HT ² (kg/m ²) - .02 AGE (years)	0.20	0.03

¹BODY WT (%) = percent ideal body weight (59).

index and age ($R^2=0.20$, $P<0.03$). A graphic representation describing the relationship between LFT and body weight adjusted for age for PREO group is presented in figure 1. No significant relationships were found between LFT and body measurements in the PREL or the POSTO groups nor between LFT and the natural log of serum levels of sex hormone binding globulin (LSHBG) in the PREO or the POSTO groups.

The relationships between the natural log of serum levels of total estrogen (LE) and body measurements in the POSTO group are described in table 3. There were direct relationships found between LE and any measure of obesity we used, e.g., body weight ($R^2=0.43$, $P<0.03$), percent of ideal body weight ($R^2=0.54$, $P<0.01$), percent body fat ($R^2=0.48$, $P<0.02$), weight and height ($R^2=0.68$, $P<0.01$) and body mass index (WT/HT^2) ($R^2=0.66$, $P<0.004$). The relationship between LE and body mass index in the POSTO group is described graphically in figure 2.

In table 4 the effect of age on the relationships between LE, age and obesity indices in the POSTO group is described. There was a significant direct relationship found between LE and age ($R^2=0.45$, $P<0.03$). This relationship is presented graphically in figure 3. In the POSTO group there were also direct relationships found between LE, body weight and age ($R^2=0.67$, $P<0.01$), between LE, percent of ideal body weight and age ($R^2=0.73$, $P<0.009$); LE, percent body fat and age ($R^2=0.68$, $P<0.01$); LE, weight, height, and age ($R^2=0.86$, $P<0.005$) and between LE, body mass index and age ($R^2=0.85$, $P<0.001$). A graph describing the relationship between LE and body mass index adjusted for age of the POSTO group is shown in figure 4. The relationships

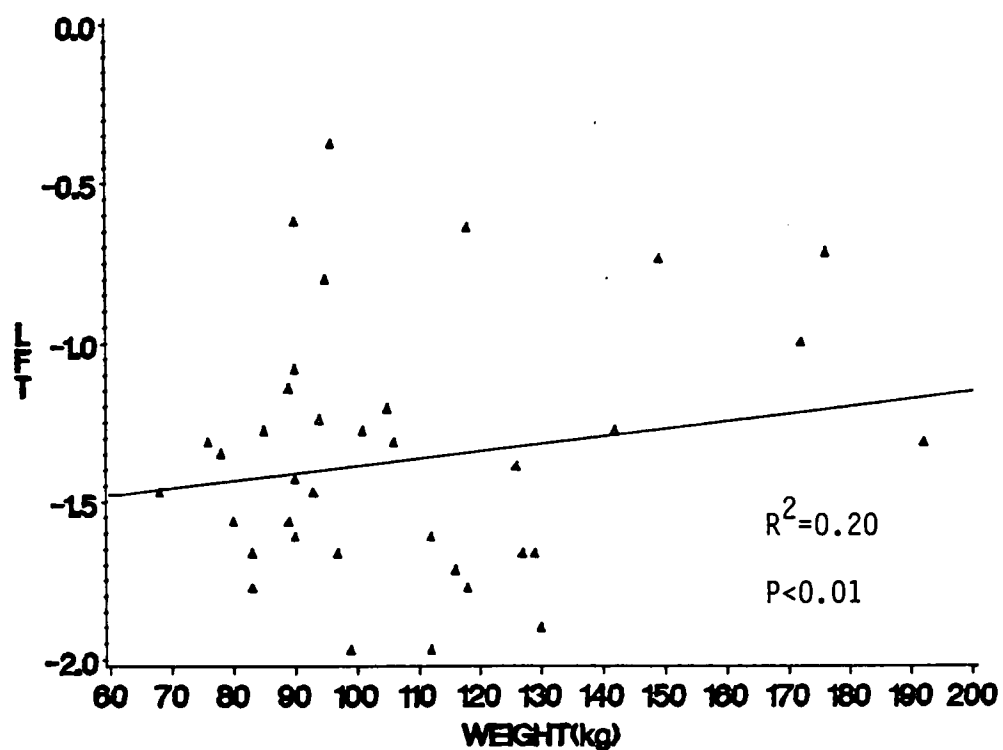


Fig. 1. Relationship of natural log of free testosterone (LFT) to weight adjusted for age (years) in premenopausal obese women (PREO). $LFT (ng/dl) = -0.94 + .003 \text{ BODY WT (kg)} - .02 \text{ AGE (years)}$; $n=38$.

Table 3. Regression Equations Describing the Relationships Between the Natural Log of Serum Levels of Total Estrogen (LE), Body Measurements in Postmenopausal Obese Women (POST0, n = 12).

EQUATION	R ²	P
LE (pg/ml) = 1.28 + 0.053 BODY FAT (%)	0.48	0.02
LE (pg/ml) = 2.26 + 0.02 BODY WT (kg)	0.43	0.03
LE (pg/ml) = 1.94 + 0.012 BODY WT (%) ¹	0.54	0.01
LE (pg/ml) = 8.00 + 0.02 BODY WT (kg) - 0.03 HT (cm)	0.68	0.01
LE (pg/ml) = 2.17 + 553.12 WT/HT ² (kg/m ²)	0.66	0.004

¹BODY WT (%) = percent ideal body weight (59).

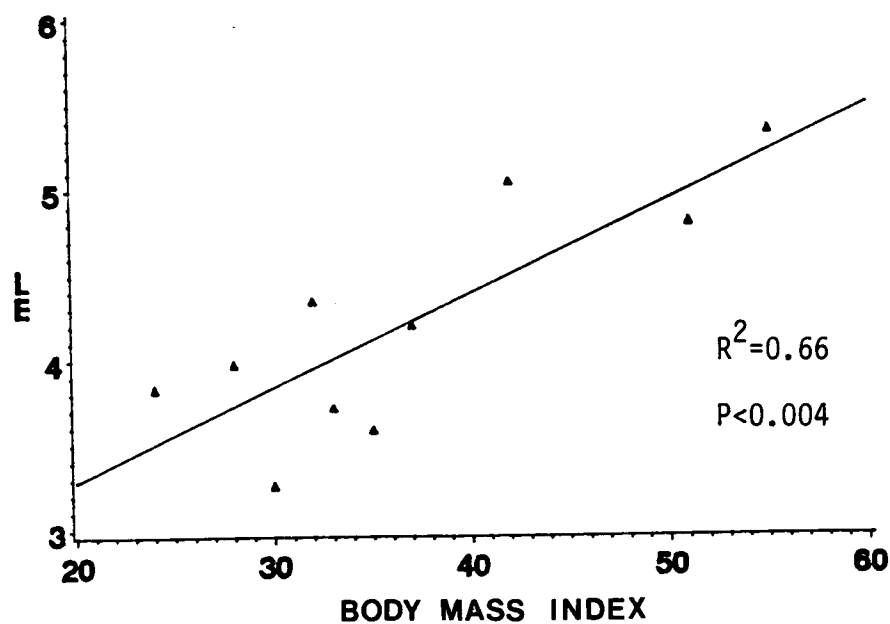


Fig. 2. Relationship of natural log of estrogen (LE) to body mass index in postmenopausal obese women (POSTO). $LE \text{ (pg/ml)} = 2.17 + 553.12 \text{ WT/HT}^2 \text{ (kg/m}^2\text{)}$; $n=12$.

Table 4. Regression Equations Describing the Relationships Between the Natural Log of Serum Levels of Total Estrogen (LE), Body Measurements and Age in Postmenopausal Obese Women (POSTO, n = 12).

EQUATION	R ²	P
LE (pg/ml) = -0.63 + 0.08 AGE (years)	0.45	0.03
LE (pg/ml) = -1.41 + 0.03 BODY FAT (%) + 0.05 AGE (years)	0.68	0.01
LE (pg/ml) = -9.6 + 0.01 BODY WT (kg) + 0.06 AGE (years)	0.67	0.01
LE (pg/ml) = -0.90 + 0.001 BODY WT (%) ¹ + 0.05 AGE (years)	0.73	0.009
LE (pg/ml) = 4.48 + 0.01 BODY WT (kg) - 0.03 HT (cm) + .05 AGE (years)	0.86	0.005
LE (pg/ml) = -0.76 + 453.04 WT/HT ² (kg/m ²) + 0.05 AGE (years)	0.85	0.001

¹BODY WT (%) = percent ideal body weight (59).

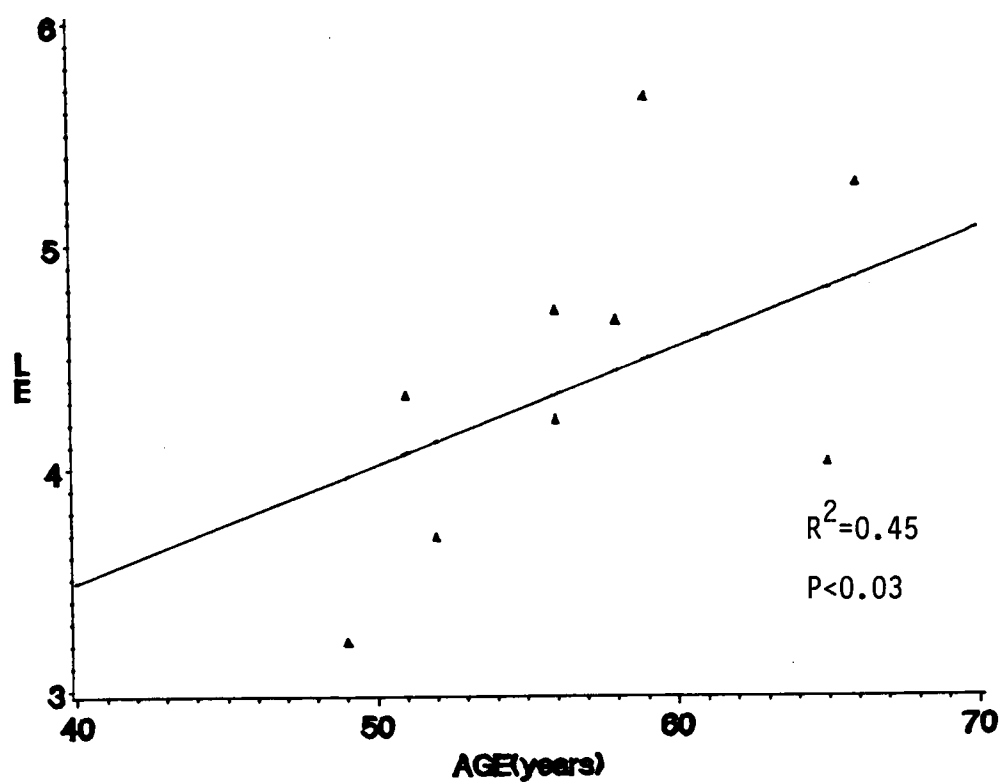


Fig. 3. Relationship of natural log of estrogen (LE) to age in postmenopausal obese women (POSTO). $LE (pg/ml) = -0.63 + 0.08 AGE (years)$; $n=12$.

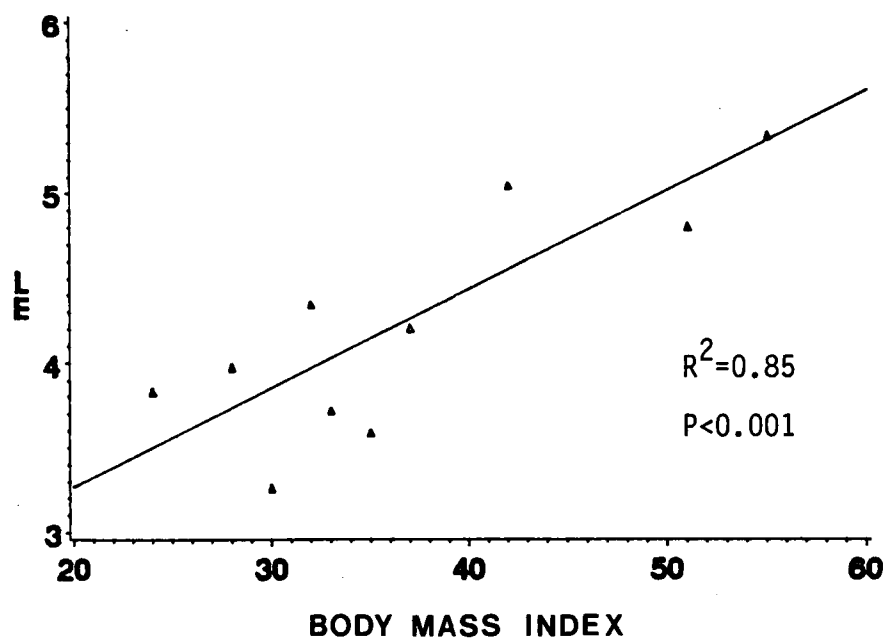


Fig. 4. Relationship of natural log of estrogen (LE) to body mass index adjusted for age (years) in postmenopausal obese women (POSTO).
 $LE \text{ (pg/ml)} = -0.76 + 453.04 \text{ WT/HT}^2 \text{ (kg/m}^2\text{)} + 0.05 \text{ AGE (years)}; n=12.$

between LE and body measurements were not significant in the PREO or PREL groups.

There was not a significant relationship observed between the natural log of serum levels of sex hormone binding globulin (LSHBG) and age or various measures of obesity in the PREO and POSTO groups. There was a low but significant ($n=29$, $R^2=0.22$, $P<0.02$) inverse relationship found between LSHBG and body weight in the PREL group (figure 5). There was also an inverse relationship found between LSHBG and body weight and height ($n=29$, $R^2=0.24$, $P<0.05$) in the PREL group. The regression equation describing this relationship was $\text{LSHBG (nmol/l)} = 6.27 - .01 \text{ HT (cm)} - .01 \text{ BODY WT (kg)}$.

There were no significant relationships found between the natural logs of serum levels of dehydroepiandrosterone sulfate (LDS) and either age or any of the body measurements in the PREO, POSTO or PREL groups.

Effect of Weight Loss on Serum Hormone

Changes in body weight observed during the study period are shown in table 5. In all, twenty-six subjects stayed in the program for 3 months and only 8 stayed in the program for the full 6 month study period. Nineteen of the 26 subjects that stayed for 3 months were premenopausal and the other 7 were postmenopausal. Of the 8 women that stayed in the program for 6 months, all were premenopausal. The change in body weight (+0.3 kg) for postmenopausal women after 3 months was not significant. The premenopausal women who stayed in the

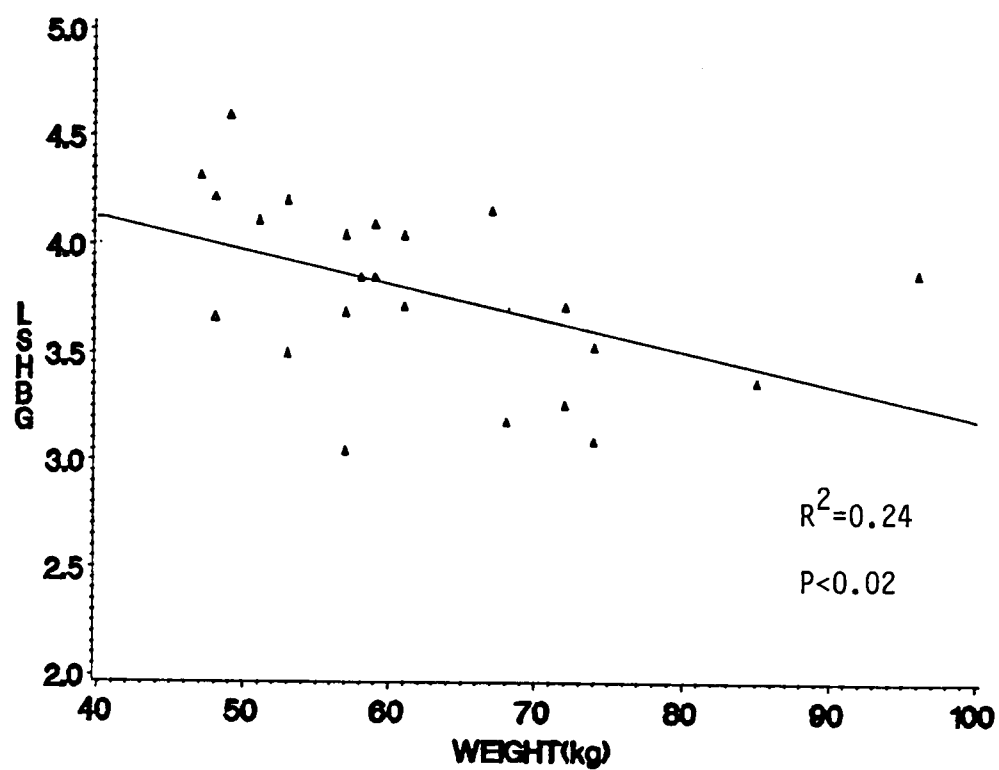


Fig. 5. Relationship of natural log of sex hormone binding globulin (LSHBG) to weight in premenopausal lean women (PREL). LSHBG (nmol/l) = $4.74 - 0.01 \text{ BODY WT (kg)}$; $n=24$.

Table 5. Initial Values and Changes in Body Weight of Premenopausal Obese (PREO) and Postmenopausal Obese (POSTO) Women Who Participated for 3 or 6 Months in the Weight Reduction Program.

	Participated for 3 Months		Participated for 6 Months	
	Initial Weight (kg)	Change in Weight (kg)	Initial Weight (kg)	Change in Weight (kg)
Premenopausal	98.3 ± 4.2 (19) ¹	-4.4 ± 1.3 (19) ²	103.4 ± 8.2 (8)	-12.8 ± 2.4 (8) ³
Postmenopausal	99.5 ± 7.5 (7)	+0.3 ± 1.6 (7)	- - - - -	- - - - -

¹Values are means ± SEM for number of subjects indicated in parentheses.

²P<0.01, paired t-test (66).

³P=0.001, paired t-test (66).

study for 3 months lost an average of 4.4 kg ($P<0.01$). For the premenopausal women who stayed in the study for 6 months, the decrement in body weight was 12.8 kg and had a high level of statistical significance ($P=0.001$).

Table 6 presents initial levels and changes in serum hormone levels of the PREO group during the weight reduction program. There were no significant changes in serum DS, E or SHBG levels in PREO women who participated in the program for 3 or 6 months. Serum FT levels were found to be significantly less ($P<0.001$) in those premenopausal women who participated in the weight loss program for the full 6 months.

Changes in serum hormone levels of the postmenopausal obese women during weight reduction are presented in table 7. No significant changes were observed in DS, FT, E and SHBG levels in postmenopausal obese women who participated in the program for 3 months; no postmenopausal women participated in the weight reduction program for 6 months.

Changes in serum hormone levels of women who experienced a marked weight loss, i.e., loss of 10 kg or more, were investigated in premenopausal obese women. No significant changes were observed in DS, E or SHBG levels after 3 or 6 months participation in the weight reduction program (table 8). The decrement in FT (-0.08 ng/dl), however, was found to be significant after both 3 and 6 months ($P<0.025$) participated in the program.

Table 6. Initial Values and Changes in Serum Hormone Levels of Premenopausal Obese (PREO) Women Who Participated for 3 or 6 Months in the Weight Reduction Program.

Hormone	Participated for 3 Months		Participated for 6 Months	
	Initial	Change After 3 Months	Initial	Change After 6 Months
DS ($\mu\text{g/dl}$) ¹	121.83 $\pm$ 13.77 (18) ²	+18.61 $\pm$ 10.16 (18)	112.25 $\pm$ 17.56 (8)	-10.00 $\pm$ 20.63 (8)
FT (ng/dl) ³	0.29 $\pm$ 0.03 (19)	-0.10 $\pm$ 0.06 (19)	0.24 $\pm$ 0.04 (8)	-0.09 $\pm$ 0.01 (8) ⁴
E (pg/ml) ⁵	220.39 $\pm$ 26.13 (18)	+2.33 $\pm$ 26.06 (18)	242.28 $\pm$ 48.74 (7)	-62.28 $\pm$ 59.09 (7)
SHBG (nmol/l) ⁶	44.81 $\pm$ 5.89 (16)	+11.37 $\pm$ 6.61 (16)	55.29 $\pm$ 9.02 (7)	-1.57 $\pm$ 7.85 (7)

¹DS = serum dehydroepiandrosterone sulfate.

²Values are means $\pm$ SEM for number of subjects indicated in parentheses.

³FT = serum free testosterone.

⁴p<0.001, paired t test (66).

⁵E = total serum estrogen.

⁶SHBG = serum sex hormone binding globulin.

Table 7. Initial Values and Changes in Serum Hormone Levels of Postmenopausal Obese (POSTO) Women Who Participated for 3 Months in the Weight Reduction Program.¹

Hormone	Initial	Change After 3 Months
DS ($\mu\text{g/dl}$) ²	83.83 $\pm$ 6.80 (6) ³	+ 9.50 $\pm$ 11.52 (6)
FT (ng/dl) ⁴	0.30 $\pm$ 0.07 (6)	- 0.06 $\pm$ 0.05 (6)
E (pg/ml) ⁵	99.43 $\pm$ 24.71 (7)	-17.00 $\pm$ 19.92 (7)
SHBG (nmol/l) ⁶	32.75 $\pm$ 8.55 (4)	- 0.75 $\pm$ 4.49 (4)

¹No POSTO women participated in the weight reduction program beyond 3 months.

²DS = serum dehydroepiandrosterone sulfate.

³Values are means $\pm$ SEM for number of subjects indicated in parentheses.

⁴FT = serum free testosterone.

⁵E = total serum estrogen.

⁶SHBG = serum sex hormone binding globulin.

Table 8. Initial Values and Changes in Serum Hormone Levels in Premenopausal Obese (PREO) Women Who Lost at Least 10 kg After Participating 3 or 6 Months in the Weight Reduction Program.

Hormone	Participated for 3 Months		Participated for 6 Months	
	Initial	Change After 3 Months	Initial	Change After 6 Months
DS ($\mu\text{g/dl}$) ¹	132.57 $\pm$ 25.07 (7) ²	+29.28 $\pm$ 17.90 (7)	108.80 $\pm$ 5.21 (5)	+ 6.40 $\pm$ 18.02 (5)
FT (ng/dl) ³	0.27 $\pm$ 0.05 (7)	- 0.08 $\pm$ 0.03 (7) ⁴	0.26 $\pm$ 0.25 (5)	- 0.08 $\pm$ 0.02 (5) ⁴
E (pg/ml) ⁵	189.42 $\pm$ 45.67 (7)	- 2.14 $\pm$ 45.37 (7)	224.80 $\pm$ 7.49 (5)	-79.60 $\pm$ 82.16 (5)
SHBG (nmol/l) ⁶	47.50 $\pm$ 9.81 (6)	+ 7.30 $\pm$ 15.99 (6)	53.00 $\pm$ 3.60 (5)	+18.40 $\pm$ 21.65 (5)

¹DS = serum dehydroepiandrosterone sulfate.

²Values are means $\pm$ SEM for number of subjects indicated in parentheses.

³FT = serum free testosterone.

⁴p<0.025, paired t-test (66).

⁵E = total serum estrogen.

⁶SHBG = serum sex hormone binding globulin.

Effect of Exercise on Serum Hormones

Mean levels of DS, FT, E and SHBG of exercising and non-exercising premenopausal obese women prior to their participation in the weight reduction program are shown in table 9. The exercising premenopausal obese women appeared to have higher serum levels of DS, E and SHBG than did the non-exercising premenopausal obese women; however, the differences were not statistically significant. No similar comparisons were made for postmenopausal obese women since only 1 of them exercised prior to participation in the weight loss program.

Initial values and changes in serum hormone levels of exercising premenopausal obese women after 6 months in the weight reduction program are shown in table 10. Only the change in FT (-0.07 ± 0.02 ng/dl) was significant. Among the 8 PREO women who stayed in the study for 6 months, there was no significant difference in the change in FT values between exercisers and non-exercisers when change in body weight was considered ($n=8$, $R^2=0.38$, $P<0.3$). Exercising PREO women lost slightly more weight (11.8 ± 8.8 kg, $n=5$) than did non-exercising PREO women (10.0 ± 2.2 kg, $n=3$); the difference in weight loss was non-significant, however. The increase in SHBG ($+25.60 \pm 14.56$ nmol/l) in exercising PREO after 6 months in the weight reduction program approached significance ($P<0.1$).

Table 9. Comparison of Serum Hormone Levels of Exercising and Non-Exercising Premenopausal Obese (PREO) Women Prior to Participation in the Weight Reduction Program.

Hormone	Exercising	Non-Exercising	Significance ¹
DS ($\mu\text{g/dl}$) ²	115.70 $\pm$ 22.00 (10) ³	108.96 $\pm$ 11.78 (27)	NS
FT (ng/dl) ⁴	0.25 $\pm$ 0.03 (10)	0.28 $\pm$ 0.02 (27)	NS
E (pg/ml) ⁵	245.88 $\pm$ 31.53 (10)	192.81 $\pm$ 24.02 (22)	NS
SHBG (nmol/l) ⁶	45.33 $\pm$ 7.10 (9)	37.18 $\pm$ 3.56 (22)	NS

¹Students t test (66).

²DS = serum dehydroepiandrosterone sulfate.

³Values are means $\pm$ SEM for number of subjects indicated in parentheses.

⁴FT = serum free testosterone.

⁵E = total serum estrogen.

⁶SHBG = serum sex hormone binding globulin.

Table 10. Initial Values and Changes in Serum Hormone Levels of Exercising Premenopausal Obese (PREO) Women Who Participated for 6 Months in the Weight Reduction Program.

Hormone	Initial Level	Change After 6 Months
DS ($\mu\text{g/dl}$) ¹	87.60 $\pm$ 17.64 (5) ²	+ 7.20 $\pm$ 9.58 (5)
FT (ng/dl) ³	0.20 $\pm$ 0.02 (5)	- 0.07 $\pm$ 0.02 (5) ⁴
E (pg/ml) ⁵	267.75 $\pm$ 33.33 (4)	-67.25 $\pm$ 49.16 (4)
SHBG (nmol/l) ⁶	48.40 $\pm$ 10.26 (5)	+25.60 $\pm$ 14.56 (5)

¹DS = serum dehydroepiandrosterone sulfate.

²Values are means $\pm$ SEM for number of subjects indicated in parentheses.

³FT = serum free testosterone.

⁴p<0.025, paired t-test (66).

⁵E = total serum estrogen.

⁶SHBG = serum sex hormone binding globulin.

CHAPTER V

DISCUSSION

The low but significant relationship between FT and degree of obesity in PREO (table 2, $P < 0.01$) is in agreement with other studies (67, 68). There are several reports of elevated serum FT levels in hirsute women (11, 12, 17) and obese women with polycystic ovarian (PCO) disease (21, 67). Friedman and Kim (39) also have reported that obesity was associated with increased FT levels in women of reproductive age, as observed in the present study. Thus elevated serum FT levels may be observed in obese women who are neither hirsute nor have PCO.

The PREO group showed a significant (table 6, $P < 0.001$) reduction (0.09 ± 0.01 ng/dl) in serum FT level after 6 months of participation in the weight reduction program (weight loss = 12.8 ± 2.4 kg). Weight loss in other studies as a result of either jejuno-ileal bypass surgery (28), gastric stapling (31, 69) or low calorie dieting (69) also was accompanied by a significant reduction in serum FT in premenopausal obese women. These results suggest that weight loss normalizes elevated FT levels in premenopausal obese women.

Exercise activity was quite varied in degree of intensity, frequency and duration among the premenopausal obese women who did exercise in the present study. The postmenopausal obese women, by and large, did not exercise. This was probably due to a combination

of their age and excessive body weight (70, 71) which made exercise, even walking, particularly uncomfortable for them. Five of the 8 premenopausal obese women who stayed in the weight reduction program exercised. These women lost more weight than did the non-exercisers (11.8 kg vs. 10.0 kg) but the difference was not significant. This finding supports the importance of incorporating exercise into weight reduction programs, as observed by others (71).

There was no significant difference in the change in serum FT between the exercising PREO (n=5) and the non-exercising PREO (n=3) when the change in body weight was considered in the analysis. This suggests that the significant decrease in serum FT levels (table 10, 0.07 ± 0.02) in the exercising PREO (n=5) women did not appear to be independent of the change in body weight experienced by these women.

Although the initial mean SHBG levels in the PREO group (39 nmol/l) appeared to be lower than that of the PREL group (table 1, 47 nmol/l), the difference only approached significance ($P < 0.1$). Decreased SHBG levels have been associated with increased FT in other studies (15, 16, 21, 22).

There was a significant inverse relationship between body weight and serum SHBG levels in the PREL group (figure 5, ($P < 0.02$)). Also, in PREO women who lost 10 kg or more during participation in the weight loss program, there was a tendency for increased SHBG levels (table 8). The lack of a significant relationship between initial body weights and serum SHBG levels in the PREO and POSTO groups cannot be explained. Decreased SHBG levels have been reported in hirsute

obese (20) and obese women with PCO (21-22). Mathur et al. showed that women who were both hirsute and obese had the lowest SHBG levels (20).

The nonsignificant relationship between body weight or other measures of obesity and serum estrogen levels in premenopausal obese women is in agreement with findings of others (35-37, 39). Cyclic obese women were found to have normal 24-hour mean plasma concentrations of estrone and estradiol by Zumoff et al. (35). The production of estrogen by the ovaries of premenopausal women may be of such magnitude that it overshadows the peripheral production of estrogen by adipose tissue even in obese women. The significant positive relationship in POSTO women (table 3) between serum LE levels and several measures of obesity, i.e., body weight ($P<0.03$), percent of ideal body weight ($P<0.01$), percent body fat ($P<0.02$), weight and height ($P<0.01$) and body mass index ($P<0.004$) is in agreement with the findings of others (38-40). Postmenopausal obese women also have a decreased risk for osteoporosis and increased risk for endometrial cancer which is possibly related to their increased production of estrogen (39).

In the present study estrogen levels were found to be associated with body fat and age in postmenopausal obese women (table 4, $P<0.01$). Jensen et al. (41) found an increase in body fat with age and a significant relationship between body fat and estrogen in postmenopausal women. In the present study the positive relationship between serum LE and various indicators of obesity in the POSTO group became even more pronounced when age was included in the model. The most

significant relationship (table 4, $P < 0.001$) between LE and the various indicators of obesity in the POSTO group was with the Quetelet body mass index (WT/HT^2) adjusted for age. The Quetelet body mass index has been shown to be highly correlated with percent body fat and other measures of obesity and is often used to describe the degree of obesity (38, 40).

Both obese men (33) and obese women (28, 31) have been reported to experience a normalization of altered hormones after weight loss. In a study of 12 obese (body weight 124%-193% of ideal) postmenopausal women, O'Dea et al. (30) reported significant reductions in serum estradiol after an average weight loss of 18 kg. Normalization of urinary excretion of estrone and estradiol was reported by de Waard et al. (38) in a study of 42 obese postmenopausal women after 12 months in a weight reduction program and a 4.5 kg average weight loss. The lack of a significant change in estrogen levels in the present study by the POSTO women is not surprising since significant reductions in their body weights were not observed.

The finding of a nonsignificant difference between serum DS levels in PREO women (table 1, 121 $\mu\text{g/dl}$) compared to PREL women (136 $\mu\text{g/dl}$) and the normalcy of these levels (61) suggests that these ovulating obese women were not hyperandrogenic. Elevated serum DS levels in overweight infertile women suspected of hyperandrogenism have been reported (17). Even in a state of androgen overproduction, an increase in the metabolic clearance rate of DS may be associated with normal levels of the hormone in the serum (52). Thus, since

metabolic clearance rates were not measured in the present study, it cannot be stated with certainty that there was not an overproduction of androgens in these women.

The significantly higher level of serum DS ($P < 0.05$) of the PREO (table 1, 121 $\mu\text{g/dl}$) and PREL (136 $\mu\text{g/dl}$) as compared to the POSTO (89 $\mu\text{g/dl}$) was probably due to the difference in ages of the pre- and postmenopausal groups. A decline in serum dehydroepiandrosterone and DS levels has been reported with age with a particularly pronounced decline following menopause (19).

Suggestions for Future Research

The results of this study have several implications for future research of this type. An increased adherence to the weight reducing regimen accompanied by an increased magnitude of weight loss with an increase in number of subjects would assist in drawing conclusions about the effects of weight loss on hormonal status. Investigating the effect of exercise on serum androgens independent of weight loss by comparing levels after weight loss by exercise or diet in two controlled obese populations would appear to be a useful study. The addition of a postmenopausal lean group would allow comparisons of postmenopausal obese women with a normal weight population. The determination of metabolic clearance rates of these hormones would create a clearer picture of the state of androgen metabolism. It would be desirable to achieve normal body weight in morbidly obese women rather than just weight loss to more thoroughly investigate the relationships

between body weight and hormone levels. To investigate the permanency of hormonal state of formerly obese populations after weight reduction, it would be of value to determine androgen levels after some period of time (e.g., after 6 months following weight loss) during which body weight was maintained.

Summary

Serum levels of dehydroepiandrosterone sulfate (DS), free testosterone (FT), estrogen (E) and sex hormone binding globulin (SHBG) were studied in 50 morbidly obese women who participated in a 6 month weight reduction program. The premenopausal obese women (n=38) who averaged 109 kg body weight at the beginning of the study and the postmenopausal obese women (n=12) who averaged 92 kg were compared to premenopausal lean women (n=29) who averaged 60 kg.

Postmenopausal obese women had significantly lower initial serum DS levels (86.8 $\mu\text{g/dl}$) than did premenopausal obese women (121.8 $\mu\text{g/dl}$) and premenopausal lean women (136.3 $\mu\text{g/dl}$). Postmenopausal obese women also had significantly lower initial serum E levels (83.1 pg/ml) than did premenopausal obese women (215.3 pg/ml) and premenopausal lean women (195.3 pg/ml). There were no significant differences observed in initial serum levels of FT or SHBG among the 3 groups.

Premenopausal obese women demonstrated a significant direct relationship between the natural log of initial serum free testosterone (LFT) and body weight adjusted for age as well as LFT and percent ideal body weight adjusted for age, LFT and percent body fat adjusted for

age and LFT and body mass index adjusted for age. These relationships were not significant in postmenopausal women.

Postmenopausal obese women demonstrated a significant direct relationship between the natural log of initial total serum estrogen (LE) and body weight, LE and percent of ideal body weight, LE and percent body fat, LE and weight and height, and LE and body mass index. These relationships were not significant in premenopausal women. When adjusted for age, the postmenopausal women demonstrated an even more significant direct relationship between LE (primarily estrone) and obesity indices, i.e., body weight, percent of ideal body weight, percent body fat, weight and height and Quetelet body mass index (WT/HT^2).

After 3 months into the weight reduction program, 19 of the original 38 premenopausal obese women remained in the study and had lost an average of 4.37 kg; after 3 months 7 of the original 12 postmenopausal obese women remained in the study and had gained an average of 0.34 kg. After 6 months into the weight reduction program, 8 premenopausal obese women remained in the study, and they had lost an average of 12.78 kg. No postmenopausal obese women participated in the study for 6 months. The premenopausal obese women who stayed in the study for 6 months ($n=8$) experienced significant reduction in FT (-0.09 ng/dl). Premenopausal obese women who exercised during the study ($n=5$) also experienced significant decreases in FT (-0.07 ng/dl), but the decrement was not independent of weight loss. Adherence to the weight reduction program by the postmenopausal obese women was not good, consequently neither weight loss or hormone changes were significant.

This study supports the theory that weight loss tends to decrease FT toward non-obese levels and increase SHBG values in obese premenopausal women. The failure of obese postmenopausal women to participate beyond 3 months in the weight reduction program with the concomitant lack of weight loss did not allow conclusions of the effect of weight loss on their hormonal status. Exercise activity appeared to aid the weight loss process and the decrease of hormone levels.

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LITERATURE CITED

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APPENDIXES

APPENDIX A

CONSENT FORM

This study performed by the University of Tennessee is being done to measure changes in serum steroid levels with weight loss. It is an accepted phenomenon that some adrenal hormones are elevated in overweight individuals and that there is an increase in reproductive infertility in some overweight individuals. The intent of this research is to see if these hormones return to normal levels with weight loss or if they remain elevated. If the later is found to be true, this may be the beginning of an explanation as to why obese individuals have difficulty losing weight.

If you decide to participate in the study, which will be indicated by your signing this consent form, you will allow the researcher access to your medical records here at the weight control unit and agree to give a small blood sample once a month for not more than six months. This blood sample (10 milliliters) will be taken by a qualified phlebotomist at one of your regular meetings and will take only a few minutes. The risk involved is considered to be minimal, that which exists anytime you donate blood. You may have a small bruise or have a little soreness around the immediate area where the blood is taken for a day or two.

Your decision to participate in this study or not will not effect your treatment at the weight control unit in any way. Furthermore, your identity will remain anonymous in any publication that would result from this research. Please ask any questions if there is anything that you do not understand.

I understand that in the event of physical injury resulting from the research procedures, the University of Tennessee does not provide monetary compensation or medical treatment as a matter of course.

I have read the description of the hormone study and I have freely volunteered to participate in it. I have had possible side effects explained to me. I have had an opportunity to ask questions and I have received acceptable answers. I understand that I may withdraw from this study at any time for any reason.

Patient's Signature

Witness

Investigator

Address

Date

Phone Number

211566
Rev. 12-79

DATE

ST. LUKE'S - ROOSEVELT
HOSPITAL CENTER
NEW YORK CITY

NEW DRUG OR PROCEDURE
CONSENT FORM

The nature and purpose of the procedure or study being carried on by the doctors at St. Luke's—Roosevelt Hospital Center, and which are described below, have been explained to me and those aspects which are new or experimental have been pointed out.

I understand that the purpose of this procedure is to develop improved methods of diagnosis and treatment but that at the present time no representation can be made that my participation will be directly beneficial to me.

Effects or hazards which might occur appear below and these and the uncertainties of new or experimental procedures generally have been explained to my full satisfaction and all of my questions have been

answered by _____

Principal Investigator

Description of study or procedure: The intent of this research project is to investigate androgen levels in the blood of obese women on a weight reducing program. Participants will allow the investigator access to their medical records at the weight control unit and donate a blood sample (1/3 ounce) once a month for no more than 6 months. The blood will be taken by a qualified blood drawer at one of the weekly discussion group meetings. A few questions concerning their menstrual cycle and exercise activity will be asked at this time.

Effects or hazards which might occur: The risk involved is considered to be minimal, that which exists anytime one donates blood. There may be a small bruise or a little soreness around the immediate area where the blood was taken for a day or two.

I understand that in the event of physical injury resulting from research procedures used in this study, only immediate essential medical treatment as determined by the Hospital will be available for the injury without charge to me personally; there will be no monetary compensation.

I voluntarily consent to participate in the study (procedure), with the understanding that I may withdraw at any time without my care being affected.

Principal Investigator

Patient's Signature

Date _____

SPECIAL INSTRUCTIONS:

In almost all cases of this nature, the patient himself, or herself, is required to sign.

As this is a consent for research or new procedure, the patient who signs must be 18 years or over.

If an exception is to be made, Hospital Administration must be consulted.

APPENDIX B

Code # _____

RECORD SHEET

NAME _____ Discussion group meeting _____
 PHONE _____ Date _____ Week of Follicular Phase ()1; ()2; ()3; ()4
 LMP Date _____ (Sample Collection)
 INITIAL WEIGHT/HEIGHT _____ Hirsutism ()0; ()+1; ()+2; ()+3; ()+4; ()+
 IDEAL BODY WEIGHT _____

	DATE/ TIME	TIME OF LAST MEAL	WEIGHT	LMP	EXERCISE FOR PAST WEEK	COMMENTS	% Dietary Fat/d
FIRST SAMPLE ()							
SECOND SAMPLE ()							
THIRD SAMPLE ()							
FOURTH SAMPLE ()							
FIFTH SAMPLE ()							
SIXTH SAMPLE ()							

VITA

Donna S. Pierce was born in Louisville, Kentucky on May 10, 1957. She attended elementary school at St. Paul in Louisville and was graduated from Angela Merici High School in May 1975.

She received a Bachelor of Science degree in Foods and Nutrition from the University of Louisville in December 1979. She continued her work as Health, Physical Education and Recreation Director for the YWCA in Louisville until fall 1980.

In August 1980 she accepted a graduate teaching assistantship in the Department of Nutrition and Food Science, The University of Tennessee, and moved to Knoxville to pursue her Master of Science degree in Nutrition Science with a minor in Exercise Physiology. After receiving her Master's degree in August 1981, she continued her assistantship and began working toward the Doctor of Philosophy degree.

In 1983 she received the General Foods Fellowship Award, the most prestigious award offered by the College of Human Ecology, The University of Tennessee, Knoxville. Later in that year she moved to New York City to gather data for her dissertation research at Columbia University Obesity Research Center. She returned to Knoxville in January of 1985 and received her Ph.D. in Human Ecology with a major in Nutrition and a minor in Exercise Physiology in June 1987. Ms. Pierce is a registered dietitian and a member of the American Dietetics Association.