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RESISTANCE EXERCISE TRAINING, HORMONE REPLACEMENT THERAPY, LEAN AND FAT MASS, AND SERUM ANABOLIC AND CATABOLIC HORMONES IN NON-OBESE AND OBESE POSTMENOPAUSAL WOMEN

by

Arturo Figueroa Gálvez

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As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Arturo Figueroa Galvez

entitled Resiatance Training, Hormone Replacement Therapy, Lean and Fat mass, and serum anabolic and Catabolic Hormones in non-obese and Obese Postmenopausal Women.

and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of

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Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copy of the dissertation to the Graduate College.

I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

Timothy M Johnson — Dissertation Director

7/7/99 — Date

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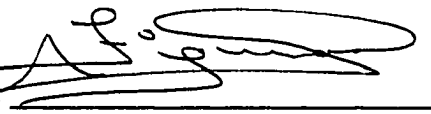
SIGNED: 

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This dissertation is a small piece of a large study originally designed to examine the effects of resistance exercise training and hormone replacement therapy on bone mineral density in postmenopausal women. Therefore, the present project which studied the effects on fat and lean soft tissue mass in addition to the determination of circulating hormonal levels would not have been possible without the cooperation, insight and efforts of Drs. Timothy G. Lohman and Scott B. Going, and the members of the BEST study. It is not possible to acknowledge everyone who contributed to this dissertation, but the following is a partial list of individuals who deserve special mention:

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DEDICATION

To my wife, Guadalupe, and our three children Arturo, Mónica and Daniela for their patience, understanding, sacrifice, and love during these years.

To my parents, José Maria and Fabiana for their love, direction and support.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

LIST OF ILLUSTRATIONS

Page

LIST OF TABLES

LIST OF TABLES (Continued)

ABSTRACT

The present study was designed to test the hypothesis that hormone replacement therapy (HRT) and exercise training would be related to differences in resting hormone levels in association with soft tissue composition changes in postmenopausal women. Estrone (E_1), estradiol (E_2), androstenedione (A-4), cortisol, growth hormone (GH) and insulin-like growth factor I (IGF-I) were determined along with estimates of lean soft tissue (LST) and fat mass in total and regional body by dual-energy x-ray absorptiometry in a cross-sectional sample of women on HRT (n=38) and not on HRT (no HRT, n=46) and in a 12 month longitudinal data of the effects of exercise training on these variables.

Postmenopausal women aged 40–65 years who were on HRT and no HRT were randomized to exercise [HRT (EX+HRT) and no HRT (EX))] and no exercise [HRT (HRT) and no HRT (CONTROL)]. Subjects were further classified in non-obese and obese (> 40% fat) resulting in the following groups: no EX (non-obese and obese) and EX (non-obese and obese).

Obese HRT had significant higher E_1, E_2, and lower GH than non-obese HRT. IGF-I was lower in obese HRT compared to both non-obese HRT and no HRT. Non-obese HRT had higher cortisol than non-obese no HRT. Exercise training decreased E_1 and E_2 with no effect on GH, IGF-I, A-4 and cortisol.

Exercise training without HRT increased total body, arms and legs LST and decreased % fat. Arm LST increased in EX+HRT and in both non-obese EX and obese EX. Leg LST and % fat increased and decreased, respectively, in non-obese EX.

The following was concluded from the study: there were no HRT effect on LST; HRT resulted in high E_1, E_2, GH, and cortisol, and low IGF-I; obesity was positively related to E_2 and negatively related to GH and IGF-I; obesity in addition to HRT was associated with a greater decrease in IGF-I; HRT had no beneficial effect on LST gains and fat mass losses resulting from exercise training; our exercise training effectively increased arm LST but not leg LST in the obese; exercise training did not modify E_1, E_2, A-4, cortisol, GH and IGF-I.

CHAPTER 1: INTRODUCTION

Explanation of the Problem and its Context

Body composition changes in normal aging individuals include loss of bone mass and lean soft tissue (LST), particularly skeletal muscle, and gain in fat mass (Forbes & Halloran, 1976). Osteoporosis, a serious health problem in postmenopausal women, is associated with the age-related decrease in muscle strength. The major determinant of the reduction in muscle strength is a loss of skeletal muscle mass (Frontera et al., 1991). The age-related loss of muscle mass, muscle strength and function (sarcopenia) may be a factor by which older persons become frail and at higher risk of fall and bone fractures.

A decrease in cross-sectional area of the thigh along with increased intramuscular fat after age 30 has been reported for men and women (Imamura et al., 1983). There is a more rapid loss of muscle mass after age 50. Although the absolute muscle mass loss with aging is more pronounced in men (Gallagher et al., 1997), the relative loss of muscle mass appears to be similar in both sexes (Going et al., 1995). Changes in the potassium/fat free mass ratio (K/FFM) show greater loss of K than FFM with age indicating a greater loss of muscle mass than FFM (Going et al., 1995). Baumgartner et al. (1995) found an average loss of 6 to 7% of LST, especially in the legs, in healthy men and women from 65 to 85 years of age.

Heymsfield et al. (1990) have proposed that appendicular skeletal mass (ASM) determined by dual-energy x-ray absorptiometry (DXA) is a good reflection of skeletal muscle mass. LST of the extremities is almost entirely composed of skeletal muscle and ASM accounts for 75-80% of total body skeletal muscle mass (Wang et al., 1996). With

aging, ASM decreases by about 9% in postmenopausal women compared with their younger counterparts (Horber et al., 1997). Baumgartner et al. (1998) have suggested that to define sarcopenia it is necessary to have a measure of muscle mass relative to height since absolute muscle mass is size dependent with height (ASM / height^2).

Concomitant with the age-related decline in LST, increases in fat mass and changes in fat distribution favor an increase in the ratio of upper-to lower-body fat in both sexes (Horber et al., 1997). Early postmenopausal women experience changes in fat distribution with an increase in abdominal adiposity (android distribution) that appear to be more related to menopause than to age (Svendesen et al., 1995; Tremollieres et al., 1996). This change in fat distribution is also observed in obese postmenopausal women who have a higher proportion of total fat mass in the trunk and lower proportion of total fat and lean mass in the legs than premenopausal women (Panotopoulus et al., 1996). Forbes (1987) found that heavier people who have higher body fat also have greater lean body mass. Although obesity in postmenopausal women has been suggested to exert a protective effect for osteoporosis since it is associated with a greater bone and LST mass and higher estrogen levels (Albala et al., 1996), increased levels of total fat mass and android fat distribution are risk factors for the development of coronary artery disease, hypertension, type II diabetes and dyslipoproteinemias (Pi-Sunyer, 1993; Kohrt & Hollozy, 1995; Tchernof & Poehlman, 1998).

Menopause is accompanied by cessation of estrogen and progesterone production by the ovaries. Although ovarian production of estrogens ceases at the time of menopause, the secretion of androgens persists. Interconversion of sex hormones in

peripheral tissues is an important contributor to their circulating levels. Ovarian and adrenal gland production of androstenedione (A-4) and testosterone (T) also decreases (Ushiroyama and Sugimoto, 1995; Hartman et al., 1997), although it has been reported that A-4 and T remain relatively stable up to 76 months after menopause (Longcope et al., 1986). Through peripheral conversion, androgens are the major source of circulating estrogen levels in postmenopausal women such that at the time of menopause, estrone (E_1) derived from the aromatization of A-4 in adipose and muscle tissue (Siiteri et al. 1973; Pasquali & Casimiri, 1993; Matsumine et al., 1986) is the principal circulating estrogen.

Increased aromatization of androgens to estrogen in adipose tissue may have clinically important consequences that may affect sex hormone levels. With more adipose tissue, aromatization of A-4 to E_1 increases by 12-15% (Kissebah et al., 1989), thus altering the estrogen-to androgen ratio (Azziz, 1989). Albala et al. (1996) also found higher levels of estrogens in obese postmenopausal women. Decreased androgen levels and increased estrogen levels that are normalized after weight reduction have also been observed in obese men (Kley et al., 1979; Parker, 1989).

In addition to the decline in estrogen levels, menopause is also associated with low circulating levels of growth hormone (GH) and its mediator of most biological effects, insulin-like growth factor-I (IGF-I) (Friend et al., 1996; Ho et al., 1987). Beginning in the third decade of life, a decrease of 14% per decade in GH secretion results in lower serum levels (Corpas et al., 1993a). This decreased secretion and resting levels of GH have been associated with estrogen deficiency since premenopausal women

have greater GH and IGF-I levels than postmenopausal women (Rudman et al., 1981; Ho et al., 1987).

Aging- and menopause- related changes in circulating hormone levels might be associated with alterations in soft tissue composition in women. Poehlman et al. (1995) found that the decrease in LST and bone mass occurs at the same time after menopause. The atrophy of the LST and the increase of the fat mass commonly observed with aging have been also associated with the decrease of GH and IGF-I serum levels (Rudman et al., 1985; Roubenoff et al., 1998; Florini et al., 1985). Moreover, the age-related decreases in sex steroid hormones, GH and IGF-I levels may promote a reduction of the anabolic processes in the skeletal muscle and an increase in fat mass accretion leading to sarcopenia and obesity.

Among other factors, alteration in circulating levels of androgens may influence body fat mass and its distribution. Testosterone is a potent anabolic hormone affecting muscle tissue growth (Kuoppasalmi & Aldercreutz, 1985; Lamb, 1975) and its decreasing basal level in aging people, especially in elderly women, is related to the sex and age-dependent differences in muscle strength and the decreasing anabolic effects on skeletal muscles (Hakkinen & Pakarinen, 1993). Although testosterone is an important anabolic hormone in men, there is no correlation in myosin heavy–chain synthesis with testosterone levels in women (Balagopal et al., 1997). Instead, this synthesis is associated with IGF-I levels in men and women. Other androgens such as dehydroepiandrosterone and A-4 may be important for this process in women.

Obesity is also associated with changes in secretion and clearance patterns of several hormones including decreased GH and increased androgens and cortisol (Seidell et al., 1990a and 1990b; Parker, 1991; Williams et al, 1984). Although an inverse association of reduced GH secretion with total fat mass and % body fat has been reported in older men and women (Rahim et al., 1998; Ruobenoff et al., 1998), this association is also observed for abdominal adiposity in near normal weight subjects (Vahl et al., 1996). In addition, central fat distribution seems to be associated with an hyperandrogenic state (Evans et al., 1983) and decreased levels of sex hormone binding globulin (SHBG) (Grenman et al., 1986; Heiss et al., 1995).

Hormone replacement therapy (HRT) has been demonstrated to be effective in decreasing the risk of osteoporosis and the symptoms associated with menopause (Christiansen et al., 1981). Oral HRT in the form of either estrogen alone or combined with progestin and transdermal HRT increases estrogen and GH, decreases IGF-I (Andersson et al., 1997; Friend et al., 1996; Moe et al. 1998) and has no influence on androgen levels (Stomati et al., 1996). Changes in cortisol levels in response to HRT use are controversial with contradictory results in the literature. An increase (Lobo et al., 1982), no change (Abraham & Moroulis, 1974), and a decrease in cortisol levels (Cagnacci et al., 1997) have been reported in postmenopausal women on HRT.

Besides hormonal changes, the etiology of the body composition changes associated with menopause includes factors such as decreased physical activity. Although sarcopenia can not be entirely explained by reduced physical activity, this process is partially attenuated in physically active older men involved in strength training but not in

those trained in aerobic activities (Klitgaard et al., 1990). Resistance exercise training may be one of the most effective means of intervention to improve the physiological alterations derived from sarcopenia. Resistance exercise has proven to be an inexpensive and effective intervention that improves neuromuscular function in young men and women (Tesch, 1988; Cureton et al., 1988; Staron et al., 1990 and 1991). The relative changes observed in strength and muscle hypertrophy due to resistance exercise training are similar in both men and women (Cureton et al., 1988; Staron et al., 1991 and 1994). Some evidence indicates that these adaptations to resistance exercise training can be induced in elderly men and women when the intensity and the length of the training are appropriate (Cureton et al., 1988; Frontera et al., 1988 and 1990; Brown et al., 1990; Charette et al., 1991; Staron et al., 1991; Morganti et al., 1995).

An increase in muscle mass in response to resistance exercise training programs has been documented in a number of studies using a variety of methods, such as muscle biopsies (Charette et al., 1991; Pyka et al., 1994a), computerized tomography (Sipila & Suominen, 1995), and DXA (Ryan et al., 1994; Brown et al., 1997; Nelson et al., 1994; Taaffe et al., 1994a; Lohman et al., 1995; Treuth et al., 1994; Chilibeck et al., 1996 and 1998; Kohrt et al., 1998). Because of substantially less radiation exposure, low cost, and availability, Wang et al. (1996) suggested that the DXA method might be a practical alternative to quantify skeletal muscle mass in vivo.

General Aims

The present study was conducted as a part of a larger study designed to assess the effects of exercise training on risk factors for osteoporosis in postmenopausal women on

HRT and in those who are not on HRT. The study was designed to test the hypothesis that exercise training and the combination of exercise training and HRT would elicit positive changes in hormone levels in association with modifications in soft tissue composition. The analyses were based on both cross-sectional data of sedentary postmenopausal women either currently on HRT (n= 38) and not on HRT (n= 46), and 12 month longitudinal effects of exercise training on these women that were randomly assigned to either no exercise (n= 48) or exercise (n= 36) groups. Measurements of serum levels of GH, IGF-I, A-4, E_1, estradiol (E_2) and cortisol were made along with the assessment of total and regional soft tissue composition. The cross-sectional analyses were conducted on the baseline data and were designed to determine the differences of exogenous administration of estrogens (for at least one year) on serum hormones and its association with body composition.

The longitudinal section of the study was designed to determine whether HRT alone, exercise training alone or the combination of these two interventions was more effective for eliciting positive changes in total and regional LST and fat mass and fat mass distribution as well as to determine the association of the changes in body composition with the circulating hormone levels at rest.

The long-term adaptations to resistance exercise training in older people, especially postmenopausal women, have not been well documented. Most of the studies have reported improved muscle adaptations to resistance exercise training, but there is a lack of information about the hormonal adaptations to this type of training. Muscle hypertrophy, improved muscle strength and balance has been found in postmenopausal women

following resistance exercise training but the effect of HRT on these adaptations is not well known.

Obesity has been associated with alterations in circulating levels of several hormones including increased levels of estrogens, androgens and cortisol, and decreased GH and IGF-I (Albala et al., 1996; Seidell et al., 1990a; Parker, 1991; Williams et al, 1984; Marin et al., 1993; Rasmussen et al., 1995). Based on the adverse effects on anabolic and catabolic hormones, obesity was considered an important factor involved on the effects of HRT and exercise training in body composition.

The present study was designed to test the hypotheses that serum levels of sex hormones, cortisol GH and IGF-I would be related to HRT and to differences in LST and body fat composition and fat distribution in postmenopausal women. These variables may be different in obese and non-obese women. Positive changes in circulating hormone levels, such as increase in GH, IGF-I and A-4 as well a decrease in cortisol serum levels at rest, would be associated with favorable adaptations in LST and fat mass and fat distribution as result of resistance exercise training. This project was designed to compare the differences in LST mass, fat mass and fat distribution between postmenopausal women on HRT and those not on HRT. In addition, changes in these variables from one year of resistance exercise training in postmenopausal women either on HRT and not on HRT were investigated. Secondary aims were to determine differences at baseline in order to control for the changes on resting levels of sex steroids (E_1, E_2 and A-4), GH, IGF-I and cortisol levels in response to exercise training in postmenopausal women with and without HRT. The aims of both the cross-sectional and longitudinal aspects of the study were expanded to

determine if obesity has an influence in hormone levels and body composition at baseline and in the adaptations to exercise training.

A Review Of The Literature

Lean Soft Tissue and DXA

Muscle mass in vivo can be assessed by both indirect and direct methods. 24-hour urinary creatinine excretion reflects muscle creatinine content and can be used for total estimate of muscle mass (Heymsfield et al., 1983). The multiscan Computed Tomography (CT) and DXA methods expose the subjects to x-rays that are attenuated depending on the composition and thickness of the tissues examined. CT provides an accurate estimation of total body skeletal muscle mass (Wang et al., 1996), although its cost and radiation dose limit widespread application (Heymsfield et al., 1983). DXA provides low radiation exposure and is useful for total body and regional assessment of bone, fat and LST mass. LST mass of the extremities (appendicular) represents a high proportion (75-80%) of total skeletal muscle mass (Wang et al., 1996). Appendicular skeletal muscle mass determined by DXA is highly correlated with estimates of muscle mass by other methods such us total body potasium ($r=0.94$, $p < 0.001$) and CT ($r=0.95$, $p < 0.001$) (Heymsfield et al., 1990; Wang et al., 1996). Because of low radiation exposure, high accuracy and precision the DXA method has become the most used method to quantify the changes in skeletal muscle mass in response to exercise training (Ryan et al., 1995; Brown et al., 1997; Nelson et al., 1994; Taaffe et al., 1994a; Lohman et al., 1995; Treuth et al., 1994; Chilibeck et al., 1996 and 1998; Kohrt et al., 1998).

HRT and Soft Tissue Composition

The decrease in LST with age and its relation to estrogen deficiency are not well established. Although HRT is commonly recommended to prevent bone mass loss, its potential effects for maintaining or increasing LST have not been clearly demonstrated (Gambacciani et al., 1997). Heiss et al. (1995) found no difference in LST between postmenopausal women not taking HRT and those taking HRT. Considering that women on HRT have higher levels of GH and that GH treatment produces increases in muscle mass in GH-deficient adults (Sartori & Narici, 1994; Hansen et al., 1995), we hypothesized that higher levels of GH would have an anabolic effect in women on HRT. The fact that GH administration to older adults produces an increase in LST mass and a decrease in fat mass partially supports this hypothesis. Welle et al. (1996) found that GH administration increased muscle mass with no change in protein synthesis in men and women aged 62-74 years. However, the increase in muscle mass with GH treatment may have been due to an increase in noncontractile protein and fluid retention because there was no change in muscle strength, hypertrophy and functional capacity that may indicate a significant effect on contractile protein synthesis (Yarasheski et al., 1995). Thus, since lower-body muscle strength and lean body mass of postmenopausal women are not influenced by HRT (Taafee et al., 1995), it appears that estrogen deficiency is not an important factor in maintaining LST mass and function in non-exercising older women.

In contrast, women taking HRT had lower % body fat and abdominal fat (Heiss et al., 1995). Several studies have suggested that estrogen administration to postmenopausal women seems to prevent fat mass increase and androgenic fat distribution (Heiss et al.,

1995; Haarbo et al., 1991; Gambacciani et al, 1997) by decreasing waist circumference (Bjorkelund et al., 1996). On the other hand, Reubinoff et al. (1995) found that HRT minimizes the shift to an android fat distribution in early postmenopausal women, but the use of estrogen and progestin neither prevents nor increases the gain in total fat mass.

Although women on HRT have higher circulating GH and less android distribution of body fat, HRT may not have an effect on LST (Andersson et al., 1997; Heiss et al., 1995). The use of GH administration as a countermeasure of decreased muscle mass has lead to varied results. In young adults GH administration does not stimulate protein synthesis but stimulates lipolysis (Copeland & Nair, 1994). In contrast, in elderly women, in whom GH levels and secretion are diminished (Pyka et al., 1992), improved protein utilization has been reported after GH treatment (Butterfield et al., 1997). In addition, GH therapy in healthy postmenopausal women decreases fat mass (Thompson et al., 1995) by 5 to 15 %; however, this reduction is not significantly different than that induced by exercise alone (Taaffe et al., 1994a; Yaraheski et al., 1995). These results suggest that increased GH by HRT may have a lipolytic effect but may not increase LST in postmenopausal women.

Exercise Training and Muscle Hypertrophy

The increase in muscle mass in response to resistance exercise training varies considerably and is dependent upon factors such as individual responsiveness, previous conditioning status, intensity and duration of the training program (MacDougall, 1992). One recent cross-sectional study of master athletes that have been training for the last 12 to

14 years, suggested that strength training but not running or swimming can preserve muscle mass and function comparable to young sedentary subjects (Klitgaard et al., 1990)

Heavy-resistance exercise training (70 - 85% of 1 repetition maximum) is an inexpensive and effective means to improve neuromuscular function, muscle mass and strength in young and elderly men and women (Frontera et al.,1988; Cureton et al.,1988; Fiatarone et al.,1990; Nelson et al., 1994; Treuth et al., 1994; Lohman et al, 1995). Two major factors, neural adaptations and muscle hypertrophy, contribute to training-induced increases in strength. Neural adaptations are responsible for strength development during the first weeks of resistance training, while muscle hypertrophy accounts for an important contribution after about 10 weeks of resistance exercise training (Frontera et al., 1998; Fiatarone et al., 1991; Charette et al., 1991; Cureton et al., 1988; Sale, 1988; Hakkinen & Pakarinen, 1992). In young women strength increases and muscle hypertrophy can be observed after only 6 weeks of resistance exercise training (Staron et al., 1991). Lohman et al. (1995) found that most of the increase in muscle mass (as assessed by DXA) took place over the first 6 months of resistance exercise training in a 18 month program for premenopausal women. Previous studies, using computed tomography (CT), have demonstrated 9 to 17% increase in cross-sectional area of the trained muscles in older men after 8 to 12 weeks of heavy resistance training (Frontera et al., 1988; Brown et al., 1990). It has been established that elderly men and women retain the capacity to adapt to heavy resistance training even in the tenth decade of life (Fiatarone et al., 1990). Interestingly, resistance training studies have shown significant increases in muscle mass and strength in postmenopausal women not on HRT (Nelson et al.,1994; Charette et al.,

1991; Ryan et al., 1995). Whether HRT combined with resistance training can enhance training-induced muscle hypertrophy and strength in postmenopausal women is not known.

Skeletal muscle hypertrophy due to resistance exercise training is a direct result of greater contractile protein as a consequence of the cell repair mechanisms (MacDougall, 1992). The balance between protein synthesis and protein breakdown determines the net gain or loss of muscle protein. Increased contractile protein synthesis or decreased rate of breakdown are the possible mechanisms by which muscle grows (Kraemer et al., 1996). A controversy exists as to whether or not body protein synthesis declines with aging (Yarasheski et al., 1993; Nair, 1995). Recently, it has been demonstrated that there is a decline in the synthesis rate of myosin heavy-chain protein that contributes to the declining muscle mass and a decreased ability to remodel muscle contractile protein in middle aged and older men and women (Balagopal et al., 1997). However, Yarasheski et al. (1993) have reported increases in muscle protein synthesis rate after 2 weeks of resistance exercise training in elderly men and women without an increase in protein breakdown rate. GH in addition to resistance exercise training, however, does not improve protein synthesis rate more than resistance exercise training alone in older men (Yarasheski et al, 1995). The synthesis rate of contractile protein is correlated to circulating levels of anabolic hormones and the regulation of these processes could be different in male and females (Balagopal et al., 1997). From these findings, it appears that muscle mass gain in response to resistance exercise training may not require the increased resting levels of GH associated with HRT in postmenopausal women.

Hormones and Exercise

Human skeletal muscle is known to be under hormonal control, and acute exercise and exercise training may cause significant changes in blood hormones (Cumming et al., 1987, Kraemer et al., 1991 and 1993,Weiss et al, 1983; Hutchinson et al., 1981; Terjung, 1979). Studies of the effects of acute exercise on hormone levels in males have shown that resistance exercise increases testosterone (T) concentration (Fahey et al., 1976; Kraemer et al., 1991; Kraemer et al., 1992) with heavy loads and no change with light loads (Guezenec et al., 1986). However, the increase in serum levels of T could be due to hemoconcentration and not to increased secretion (Kraemer et al., 1992; Bunt, 1986). Most studies have not shown increases in serum T levels in females following resistance exercise or training (Westerlind et al., 1987; Kraemer et al., 1991; Hakkinen et al., 1990). However, it seems that in young females the basal T levels may be important for strength increases, even if there is no significant change in muscle mass (Hakkinen et al., 1990). Only one study has reported that strength training in young women produced an increment in resting levels of T and a small increase in T and cortisol during exercise (Cumming et al., 1987). The authors suggested an adrenal origin of the T.

Although androstenedione (A-4) is a less potent androgen than T, it may be important in women. A-4 levels are higher than levels of T in women, and their resting and post-exercise levels are higher than in men (Weiss et al., 1983). Acute increases in response to heavy resistance exercise in A-4 levels have been observed in young females (Kraemer et al., 1995a; Weiss et al., 1983). Thus, a similar A-4 response to resistance

exercise in both sexes coupled with higher resting levels of this androgen may contribute to muscle hypertrophy in women in response to prolonged heavy resistance training.

Due to its catabolic effects, cortisol levels have been considered important in the study of the hormonal responses and adaptations to resistance exercise. Furthermore, a cortisol level has been used as an indicator of overtraining (Kraemer et al., 1987; Hakkinen et al., 1985). Heavy resistance exercise that produces increases in anabolic hormones at the same time stimulate increases in cortisol (Kraemer et al., 1987, 1990, 1991 and 1996).

The lack of exercise-induced increases in T levels in women could suggest that other anabolic hormones, like GH and insulin-like growth factors (IGFs), may participate in muscle adaptations to resistance exercise training (Kraemer et al., 1991 and 1993). Most studies indicate that the anabolic effects of GH on skeletal muscle are expressed through IGFs (Friend et al., 1996; Ho et al., 1987), but growth promoting mechanisms in response to heavy resistance exercise that produced high increases in GH in men may not be related to stimulation of IGF-1 (McBride et al., 1995; Kraemer et al., 1995b). GH levels do not increase to the same level with all resistance exercise protocols. Using an intensity of 10 repetition maximum (RM) with a rest period of one or two minutes between sets and exercises in young men and women, significant increases in GH were observed, while T only increased in men (Kraemer et al., 1991; Kraemer et al., 1992). These results suggest that in resistance exercise training a threshold for intensity may exist in order to have a significant hormonal response of the hypothalamic-pituitary axis (Kraemer et al., 1996). Heavy resistance exercise at 10 RM leads to a significant increase in GH levels in young and middle-aged men and women (Kraemer et al., 1992, Kraemer et al., 1991; Hakkinen

& Pakarinen, 1995). This GH response to the same relative heavy resistance exercise is greatly decreased in elderly men and women (Pyka et al., 1992). However, three out of six subjects of the older group showed a significant GH response to a load of 85% of 1 RM (Pyka et al., 1992). These results suggest a great individual variability and a possible significant GH response to heavy resistance exercise in some elderly individuals. Obesity is an important factor to be considered because GH secretion is decreased after administration of GH-releasing factor and insulin-induced hypoglycemia in obese people (Williams et al., 1984). It has been recently been demonstrated that the age-associated decrease in LST mass can be prevented by heavy exercise training program, due at least in part to the exercise-induced increase in GH secretion (Horber et al., 1996).

It is believed that T and GH resting levels might not change after resistance training for up to 12 months in length in young (Guezennec et al., 1986; Hakkinen et al., 1985; Hetrick & Wilmore, 1977; Kraemer et al., 1998) and older men and women (Hakkinen & Pakarinen, 1994; Pyka et al., 1994b). Although no significant changes in T, cortisol and E_2 have been found in young women after three to sixteen weeks of resistance training (Hakkinen et al., 1990; Hakkinen & Pakarinen, 1992; Hetrick & Wilmore, 1977), previous studies have found high resting levels of A-4 in young competitive female swimmers (Constantini & Warren, 1995) and athletes participating in anaerobic sports compared to athletes involved in aerobic activities (Hutchinson et al., 1981). A higher muscle strength required in these sports may suggest an association between muscle mass and circulating A-4 levels in women. Some studies have demonstrated changes in resting levels of cortisol that enhanced the testosterone/cortisol

ratio during the course of 8 to 24 weeks of resistance training in young men and women (Hakkinen et al., 1985; Hetrick & Wilmore, 1977; Kraemer et al., 1998) and elderly women (Hakkinen & Pakarinen, 1994). On the contrary, when the training programs have been too stressful and inadequate recovery between the sessions is allowed, low levels of T and high levels of cortisol could lead to an overtraining state that might compromise muscle adaptations (Hakkinen et al., 1987 and 1989; Kuoppasalmi & Aldercreutz, 1985). Pyka et al. (1994b) have suggested that GH and IGF-I do not change after one year of resistance training in a group of 8 elderly men and women. However, use of both sexes with a small number of subjects and a large individual variation are factors that may have affected the results. Thus, chronic alterations in A-4, cortisol IGF-I and GH levels may be important for muscle adaptations to resistance training in postmenopausal women. Additional studies are needed to determine the effects of very prolonged resistance training, HRT and their interaction with GH, IGF-I, A-4, E_1, E_2, and cortisol in postmenopausal women.

HRT, Exercise Training and Soft Tissue Composition

Although several prospective studies have tested the efficacy of resistance exercise training in augmenting LST in postmenopausal women not on HRT, only a few training studies have been conducted combining exercise and HRT in postmenopausal women (Brown et al., 1997; Kohrt et al., 1998; Thompson et al., 1998). Kohrt et al. (1998) examined the effect of 9-month weight-bearing exercise training and HRT on body composition in elderly postmenopausal women. They found that HRT alone and HRT plus exercise attenuated fat accumulation with no effect on LST. In contrast, Brown

et al. (1997) reported that 11 month weight-bearing exercise training alone and in combination with HRT increased leg LST. However, the effects of resistance exercise training in combination with HRT on LST may be different from those found with different types of exercise training. Whether HRT combined with resistance exercise training can enhance training-induced muscle hypertrophy and fat mass reduction in postmenopausal women is not well known.

Furthermore, the effects of exercise training alone or combined with HRT on serum hormone levels and body composition in obese postmenopausal women have received little attention. In a study by Thompson et al. (1998), obese postmenopausal women (42.0 ± 5.9 % body fat), most of them on HRT, were randomly assigned to GH, IGF-I, GH+IGF-I and placebo group. After 12 weeks of diet and exercise (endurance + resistance) training program, the GH group but not the placebo group had a significant increase in FFM. Although HRT increases GH levels in non-obese women, these levels remain low in obese women (Williams et al., 1984). The results of Thompson et al. (1998) suggest that low circulating GH levels in obese postmenopausal women may limit the gains in FFM after exercise training while GH administration promotes significant gains in FFM. The study by Thompson et al. (1998) supports the contention that skeletal muscle adaptations to resistance exercise training may require an acute rise in the secretion of GH which is blunted in elderly and obese people. Inclusion of obese and non-obese subjects in the same training groups makes a study hard to interpret. Baseline differences in body composition and hormone levels in obese individuals are important factors involved in the adaptations to resistance exercise training because greater body fat

is typically associated with higher LST and estrogen levels, but also with lower GH levels.

Explanation of Dissertation Format

The present dissertation was prepared in manuscript format. Therefore, chapter 2 contains the general methods used in the present study and also a summary of the main findings and conclusions of each of the two manuscripts that are presented in chapters 3 and 4. The data presented in this dissertation is part of a larger study (BEST). Thus, my original contribution to each of the two manuscripts was the assessment of serum GH, A-4, E_1, E_2, and cortisol levels and determining the cross-sectional and longitudinal relationship to HRT use, levels of obesity, total and regional soft tissue mass, and fat distribution using DXA technology. The quality control assessment of the soft tissue and serum hormone levels in this dissertation is presented in chapter 2. The data of this dissertation have not yet been published or submitted for publication.

CHAPTER 2: PRESENT STUDY

Methods

The methods used in the present study are described below. The main findings and conclusions of the two manuscripts presented in chapters 3 and 4 are summarized at the end of this chapter.

Subjects

A total of 93 women were recruited by flyers and advertisements in newspaper and TV from the Tucson area to participate in a study designed to study the effects of exercise and HRT in two population of postmenopausal women on bone mineral density. Data from the women recruited in the first two cohorts (n=84) were included in this dissertation.

Postmenopausal women (three to eleven years after natural or surgical menopause) who were 40- 65 years old and previously sedentary (less than 2 hours of exercise per week) for at least one year were included in the study if they fulfilled all other criteria. Women taking HRT and women not taking HRT agreed to remain in the same status throughout the year of the study. Women were excluded if they were smokers, had been on HRT for less than a year or more than 3.9 years, had osteoporosis assessed by DXA scan of spine or hip (BMD $\geq$ 3 SD), had cancer treatment during the last 5 years, or were unwilling to maintain the HRT status or to be randomly assigned to the non-exercise or the exercise group. A physical exam excluded subjects who had musculoskeletal conditions that contraindicated exercise training. Aerobic capacity and cardiovascular disease were assessed by a graded treadmill stress test supervised by a

physician. Subjects accepted in the study agreed not to change their dietary habits or to try to lose weight. Those subjects in the non-exercise groups also agreed not to change their physical activity habits. All subjects also agreed to supplement their diets with 800 mg of calcium citrate daily. Informed, written consent was obtained from each subject prior to the beginning of the study. All the procedures were approved by the University of Arizona's Human Subjects Committee.

Design

Women who were either taking HRT (2.3 ± 0.8 yr, n = 39) or not taking HRT (no HRT, n = 54) were recruited into the study. A total of 93 were enrolled at the beginning of the study. Of these, 87 completed the full examination. Sample size was further reduced for analyses because of additional missing data. For the present analysis 3 subjects were deleted because of high serum levels of estrogens, suggesting they were not menopausal. Of the 38 women on HRT, 10 were on unopposed oral estrogen [Conjugated equine estrogens (CEE; n=8), micronized estradiol (E_2 , n=2)], 23 were on oral estrogen [CEE (n=18), micronized E_2 (n=3), and estropipate (n=2)] plus progestin (E-P), 2 were on oral micronized E_2 plus testosterone, and 3 were on transdermal estrogen plus progestin. Sixty one women had undergone natural menopause and twenty three had undergone surgical menopause [hysterectomy with unilateral (n=3) or bilateral (n=10) oophorectomy]. Low serum levels of E_1 and E_2 confirmed the three women with unilateral oophorectomy as postmenopausal. In cross-sectional analyses (chapter 3) we used the baseline measurements of serum hormones and body composition of women taking HRT (n=38) and not taking HRT (n=46). In order to analyze the potential influence of obesity

on hormone levels, further classification based on body fatness into obese (n=34) and non-obese (n=50) groups resulted in the following distribution: a) non-obese no HRT (n=27); b) non-obese on HRT (n=23); c) obese no HRT (n=19); and d) obese on HRT (n=15). Subjects were classified as obese with % fat > 40% as determined by DXA.

Longitudinal analyses (chapter 4) with the same eighty four previously sedentary postmenopausal women who participated in a 12 month study were designed to examine the effects of exercise training and HRT on soft tissue and some serum hormones involved in the anabolic and catabolic processes. The subjects were randomized into either exercise (n=36) or no exercise (n=48) groups resulting in the following subgroups: a) exercise plus HRT (EX+HRT; n=16); 2) no exercise on HRT (HRT; n=22); 3) exercise not on HRT (EX; n=20); and 4) no exercise no HRT (CONTROL, CONT; n=26). To analyze the potential influence of obesity on the hormone level and body composition changes to exercise training, the following subgroups were studied: a) obese EX (n=14); b) obese no EX (n=20); c) non- obese EX (n=22); and d) non-obese no EX (n=28).

Exercise Training

The supervised training program was conducted three times per week on alternating days and consisted of both resistance and weight-bearing aerobic exercises. The resistance exercises included the leg press, squat, lateral pull down, seated row, back extension, military dumbbell press and rotary torso. Workouts consisted of 2 sets of 6-8 repetitions with 45 to 60 sec of rest between sets for each exercise, alternating between medium (70% 1 RM) and high intensity (80% 1 RM) days. The 1 RM determinations were performed

every 6 weeks to progressively increase the resistance and maintain the training intensity throughout the study.

Weight-bearing exercise training consisted of warm up walking (5 min), stepping/stair climbing with weighted vest (10 min) and walking with weight vest (10 min). Walking was combined with skipping, jogging, hopping and jumping. These activities were performed at an intensity of 50-80% of maximal heart rate. The load in the weighted weight vest was progressively increased from 4 to 14 kg.

Strength Testing

Strength testing was performed on all exercise subjects to assess changes in muscle strength in selected muscle groups. The dynamic muscle strength was determined for muscles of the thigh, back and shoulders using the one repetition-maximum (1 RM) test. 1 RM strength represents the maximum amount of weight that a subject can lift once using proper technique. The exercises tested were the leg press, squat, lateral pulldown, row and military press. Only 1 RM at baseline, and changes at 6 and 12 months were used in the analysis. Percent changes at 6 and 12 months in muscle strength were calculated as follow: % change = [(6 or 12 month value – baseline value) x 100] / baseline value.

Aerobic Capacity

Oxygen consumption (VO_2) was used as a measurement of aerobic fitness at baseline and 12 months. VO_2 max was determined using an on line computer-assisted open circuit spirometry system (Vista mini-CPX, Vacu.Med, Ventura, CA). VO_2 max was reached when at least two of the following criteria were met: a plateau in VO_2 with

increasing workload, a respiratory exchange ratio > 1.10, and a heart rate within 10 beats/ min of age-predicted maximal heart rate. Peak oxygen consumption (VO_2 peak) was considered as the greatest value of VO_2 when a plateau was not reached. VO_2 max or VO_2 peak were determined with a modified Balke protocol by using a treadmill (Q65, Quinton, Seattle) with constant speed at 3.3 miles/hr. The initial grade was set at 0% for the first minute after which it increased 2% every minute until minute 14. Grade increased 1% from 24 to 25% during minute 14 and remained constant for the duration of the test. Heart rate was monitored (Q3000, Quinton, Seattle) continuously and blood pressure and rate of perceived exertion were recorded in every stage. The test was terminated when the subject was unable to continue or when the physician decided to stopped it due to contraindications to continue exercising.

Anthropometry

Barefoot standing height was measured in duplicate to the nearest 1.0 millimeter using a wall- mounted stadiometer. Body weight was obtained in duplicate to the nearest 0.1kilogram (kg) using an Accu-weigh Model 150 TK/A-58 beam scale (Metro Equipment Corp., Sunnyvale, CA). Body mass index (BMI) was calculated as the weight in kilograms divided by the square of standing height expressed in meters. Waist and hip circumferences were measured in duplicate with the subjects in standing position to the nearest 1.0 millimeter using a flexible steel tape and following standard procedures (Lohman et al., 1988). The average of two measurements was used to obtain the waist-to-hip ratio (WHR). WHR was used to estimate body fat distribution by anthropometry.

Body Composition

Women were scanned on medium speed using dual-energy x-ray absorptiometry (DXA) scanner model DPX-L (Lunar Radiation, Madison, WI). Whole body soft tissue composition was estimated from DXA with the manufacturer's software version 1.3Y. The system software divides pixels first into bone mineral (BM) and soft tissue mass. The soft tissue is then further separated into fat and lean soft tissue (LST) mass. LST (kg) was calculated as soft tissue mass (kg) minus fat mass (kg). Total body was segmented to obtain estimates of arms, legs, and trunk LST and fat mass. DXA-derived body composition variables in the two manuscripts included total body and regional fat mass and lean soft tissue mass. Total body % fat was calculated by dividing fat tissue (g) by the sum of total soft tissue (bone-free lean tissue plus fat tissue, g) and total bone mineral content (BMC, g) multiplied by 100. Body fat distribution by DXA was estimated using the trunk/legs fat mass ratio. Each subject was scanned a second time within a week at each measurement interval to assess reliability of DXA measurements and to improve precision. The precision of body composition (technical error), expressed as a coefficient of variation (CV), was determined from repeat scans (n = 2) on all subjects. The CV for fat and LST mass were 3.3 and 2.2% (total body), 9.8 and 3.5% (arms), 4.7 and 2.9% (legs), and 3.6 and 2.9% (trunk), respectively (Table 1). The average of the two scans was used in subsequent analyses.

Table 1. Coefficients of variation of DXA-determined variables for total body and regional soft tissue composition (n=84).

	Day 1	Day 2	TE	CV (%)	r	t
Fat Mass (kg)						
Total Body	26.7	27.1	0.88	3.3	0.99	-2.71
Arm	2.4	2.4	0.24	9.8	0.94	-1.10
Leg	10.6	10.7	0.50	4.7	0.98	-1.93
Trunk	12.8	13.0	0.47	3.6	0.99	-2.21
LST (kg)						
Total Body	38.9	38.8	0.85	2.2	0.96	1.32
Arm	3.8	3.6	0.13	3.5	0.97	2.45
Leg	12.6	12.6	0.37	2.9	0.95	1.16
Trunk	19.6	19.5	0.57	2.9	0.94	0.94

Technical error (TE) = squared root of [sum of (day 1 - day 2)2] / 2 (n)
Coefficient of variation (CV)= (TE/mean) * 100

Serum Collection

All blood samples were obtained from an antecubital vein after 8-12 hour fast. Following standard venipuncture procedures by a certified phlebotomist, whole blood was collected into serum vacutainer tubes and allowed to clot at room temperature for 30-120 min. After centrifugation of whole blood samples for 15 min at 3000 rpm, serum samples for hormone analyses were kept frozen at -80° C until assayed. Collections were at a similar time of the day (between 0600 and 0900 h) for baseline, 6 and 12 months to reduce the effects of diurnal variations on hormone levels. Blood draws in the exercisers were made 24-48 hr after the last exercise training session to avoid a possible acute effect of exercise on hormone levels.

Hormone Analyses

Serum levels of estrone (E_1), estradiol (E_2), androstenedione (A-4), growth hormone (GH) and cortisol were determined by radioimmunoassay (RIA) methods. The E_1 and E_2 kits were obtained from Diagnostic Systems Laboratories (Webster, TX). Serum fasting E_1 and E_2 concentrations were determined in duplicate from 50 and 200 uL aliquots of serum, respectively, using double antibodies RIAs. The standards, controls and unknown samples were sandwiched between the first and second I-^{125} labeled antibody. After decanting, the antibody-bound hormone was counted.

Serum fasting A-4 and cortisol levels were determined in duplicate from 50 and 25 uL aliquots, respectively, using commercially available coated-tubes kits from Diagnostic Systems Laboratories (Webster, TX) for A-4 and ICN Biomedicals (Costa Mesa, CA) for cortisol. In these RIAs, the antibody is covalently bound to the inner surface of

polypropylene tubes. Thus, the antibody-hormone-antibody ($I-^{125}$) complex was also bound to the tube wall. Decanting was used to leave only the antibody-bound hormone and the tubes were counted.

Serum GH levels were measured by the avidin coated beads with two antibodies RIA kit obtained from Nichols Institute Diagnostics (San Juan Capistrano, CA). The two anti-GH antibodies bind two separate antigenic determinants on the GH molecule forming a soluble sandwich complex. One of the antibodies is radiolabeled ($I-^{125}$) for detection while the other antibody is coupled to biotin. The avidin coated bead binds the sandwich complex to a solid phase via the high affinity interaction between biotin and avidin. After an overnight incubation, the bead was washed to remove unbound components and the radioactivity bound to the bead was counted.

Immunoradiometric assays were performed to determine serum levels of IGF-I. The kits were purchased from Diagnostics Systems Laboratories (Webster, TX). Using antibody coated tubes, the analyte in the standards, controls and unknown samples were sandwiched between the "anchored" antibody and a second $I-^{125}$ labeled antibody. Buffer was used to wash any unbound labeled antibody and the coated tube was counted.

A dose response curve of radioactivity vs. concentration was generated from the standard concentrations using Immunofit EIA/RIA software version 4.0 (Beckman Instruments, Fullerton, CA) for E_1, E_2, A-4 and IGF-I. Concentrations of the unknowns were determined from these curves. The Micromedic 4/200 plus (Micromedic Systems, Huntsville, AL) and isodata software (ICN Biomedicals, Costa Mesa, CA) were used to

derive cubic spline standard curves which enabled determination of cortisol and GH levels form measurements of antibody-hormone in the unknown samples.

For each hormonal assay, the low and high controls were used to determine intraassay and interassay variability. The coefficient of variation (CV) was calculated as the (standard deviation/mean) x 100. The intraassay variance (precision) was calculated from the mean of replicate determinations on the lowest and highest quality controls in a single assay for cortisol and GH. Intraassay variation for E_1, E_2, A-4 and IGF-I was evaluated using the duplicate tubes for the low and high controls in each assay. The intraassay CVs for the low and high controls were 8.7 and 11.1%, 6.9 and 5.3%, 5.6 and 8.2 %, 8.9 and 3.4%, 4.4 and 2.1 %, and 0.03 and 19.4 for E_1, E_2, A-4, cortisol, GH and IGF-I, respectively (Table 2)

The interassay variance (reproducibility) was obtained from the mean of the low and high quality controls in subsequent assays. The interassay CVs for the lowest and highest quality controls were 3.3 and 9.8%, 3.1 and 9.0, 6.5 and 9.4%, 5.7 and 11.9%, 3.6 and 8.0%, and 4.7 and 8.0% for E_1, E_2, A-4, cortisol, GH and IGF-I, respectively (Table 2, Figures 1 and 2).

Baseline, 6 and 12 month samples from each subject were processed in duplicate in the same assay to minimize inter-assay variability. Also, samples from the four groups of study were analyzed in the same assay. All samples with CVs between duplicates higher than 10% or with values below and above the lowest and highest standard were reassayed.

Table 2. Variation for the low and high controls for each hormone.

Hormone	control	expected range	Interassay			Intraassay		
			mean	sd	CV (%)	mean	sd	CV (%)
Estrone (pg/mL)	low	35 +/- 15	36.92	1.21	3.3	36.99	3.2	8.7
	high	300+/- 70	306.80	29.96	9.8	308.3	34.08	11.1
Estradiol (pg/mL)	low	20+/-5	19.72	0.61	3.1	19.74	1.37	6.9
	high	250+/-100	259.00	23.31	9.0	258.9	13.79	5.3
Androstenedione (ng/mL)	low	1 +/- 0.3	1.08	0.07	6.5	1.09	0.6	5.6
	high	6 +/- 2	6.58	0.62	9.4	6.74	0.56	8.2
Cortisol (ug/dL)	low	2.2-4.6	4.3	0.24	5.7	3.99	0.35	8.9
	high	28.7-38.3	37.8	4.53	11.9	37.46	1.29	3.4
Growth Hormone (ng/mL)	low	2.2-3.2	2.87	0.10	3.6	2.94	0.13	4.4
	high	8.6-12.9	11.85	0.95	8.0	11.76	0.25	2.1

Coefficient of Variation= CV; Standard Deviation= sd.
CV= [sd / mean] * 100

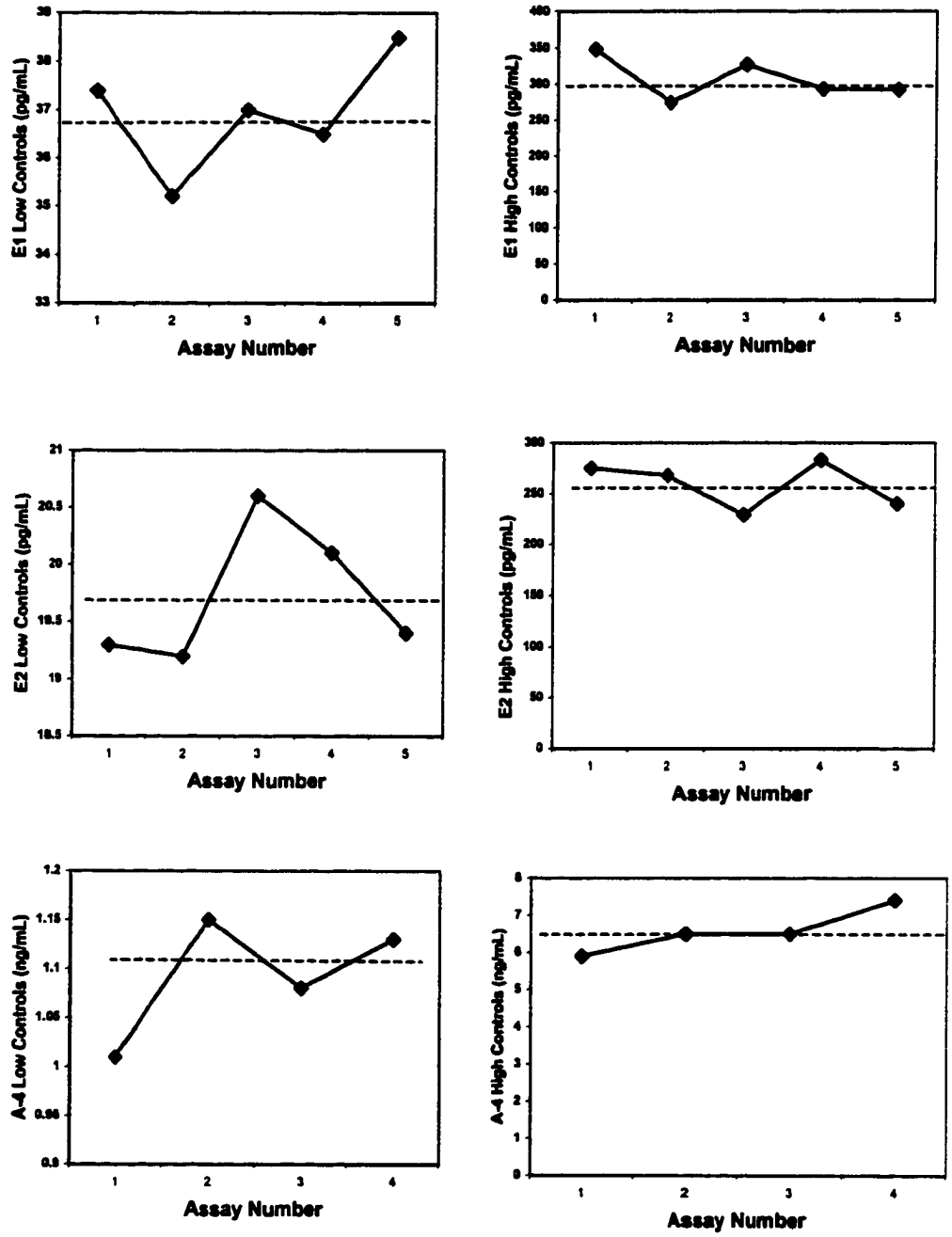

Figure 1. Low and high control values for Estrone (E_1), Estradiol (E_2) and Androstenedione (A-4) assays.

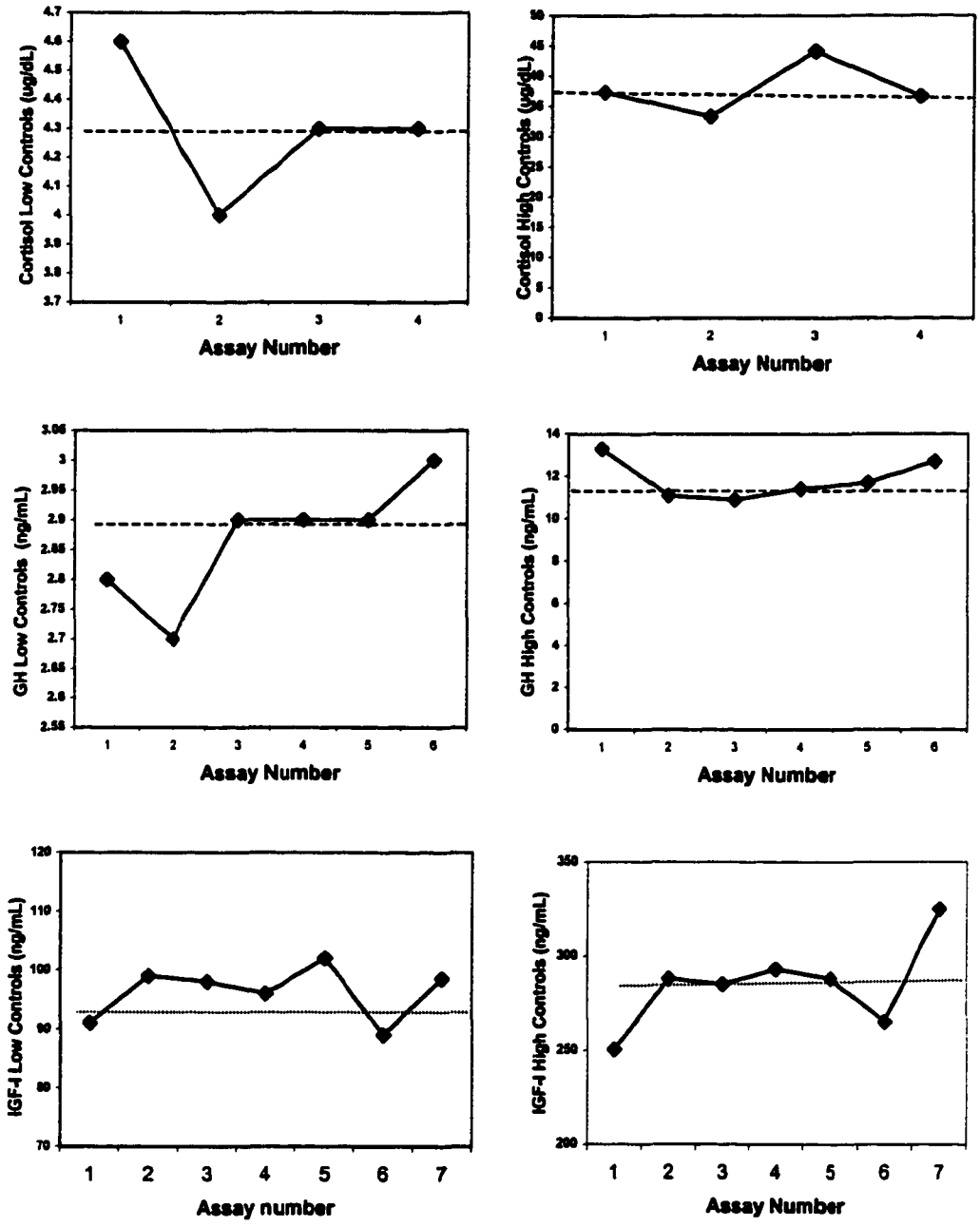

Figure 2. Low and high control values for Cortisol, Growth Hormone (GH) and Insulin-Like Growth Factor I (IGF-I) assays.

Main Findings

First manuscript: Body Composition, Steroid Hormones Growth Hormone And Insulin-Like Growth Factor I In Postmenopausal Women On Hormone Replacement Therapy.

We examined the effects of HRT on DXA- and anthropometry-derived body composition and the relationship of these assessments with serum E_1, E_2, GH, cortisol, and A-4 levels in a sample of 84 postmenopausal women with and without HRT. No significant differences between the HRT and the no HRT groups were observed for body weight, height, body mass index (BMI), WHR and all body composition variables assessed by DXA at baseline. Significantly ($p < 0.05$) higher levels of E_1, E_2, GH and cortisol were found in the HRT group as compared to the no HRT group. Because obesity modifies the levels of these hormones, subjects were classified by DXA-determined % Fat (≥ 40 vs < 40) as obese [total n=34; on HRT (n=15); no HRT (n=19)] or non-obese [total n=50; on HRT (n=22); no HRT (n=28)]. In obese women, we found greater values in weight, BMI, LST mass (total body, arms, legs, and trunk), fat mass (total body, arms, legs, and trunk), leg LST/ fat ratio and % body. The non-obese groups had lower trunk and leg fat mass than the obese groups. The obese no HRT group had significantly higher WHR than both the non-obese on HRT and non-obese no HRT. In the women taking HRT, the obese group had significantly higher levels of E_1, E_2, and lower levels of GH than the non-obese group. IGF-I levels were significantly lower in the obese women on HRT compared to both non-obese on HRT and no HRT. The non-obese on HRT group had significantly higher levels of cortisol compared to the non-obese no HRT group.

Multiple regression analyses revealed that HRT use is associated positively with E_1, E_2, GH and cortisol levels but negatively with IGF-I levels. Both E_2 and A-4 were positively associated with trunk fat mass. Obesity and trunk fat mass were inversely correlated with E_1 and E_2 levels. Both indices of fat distribution (WHR and trunk/leg fat ratio) were inversely correlated with IGF-I but not with GH levels.

Second Manuscript: Effects Of Resistance Exercise Training And Hormone Replacement Therapy On Lean Soft Tissue And Fat Mass, Anabolic And Catabolic Hormones In Postmenopausal Women.

In the second manuscript we examined the effects of 6 and 12 months of resistance training on changes in DXA- and anthropometry-derived estimates of LST and fat mass, and fat distribution and their relation with changes in serum E_1, E_2, GH, IGF-I, cortisol, and A-4 levels. Exercise training without HRT resulted in significant increases in LST of the total body (2.4 and 1.6%), arms (4.8 and 4.4%), legs (1.8 and 1.0%), and trunk (2.6 and 1.7%) at 6 and 12 months. In the EX+HRT group, the only significant increase in LST was in the arms, at 6 months. Significant decreases in % fat were observed in the EX group at 6 (-2.0%) and 12 months (-1.8%). The leg LST/Fat ratio was increased by exercise training in both EX and EX+HRT groups at 6 months. Regardless of exercise status, significant decreases in E_2 levels at 6 and 12 months, and in GH levels at 12 months were found in both no HRT groups. In the EX+HRT group, exercise training resulted in significant decreases in E_1 and E_2 at 6 months. No significant changes were observed in GH, A-4 and cortisol levels in response to exercise training.

Both obese groups (non-EX and EX) had significant decreases in leg LST at 6 months and in leg fat mass at 12 months compared to the non-obese groups. The leg LST/Fat ratio increased significantly in both obese groups at 12 months. LST in total body increased significantly in the non-obese EX (2.5%) at 6 but not at 12 months (1.4%). The obese EX group experienced significant increases in total body LST at 6 (1.6%) and 12 months (1.5%). Significant increases in arm LST were observed in both the non-obese EX (5.4%) and obese EX (5.5%) groups at 6 months, but increased significantly only in the obese EX (6.2%) at 12 months. The non-obese EX group increased significantly the LST of the legs at 6 (2.7%) and 12 months (1.5%). Trunk LST increased significantly only in the obese EX group at 6 months (3.1%). In the non-obese EX group, there was a significant increase in leg LST/Fat ratio at 6 (4.1%) and 12 months (7.8%). There was a significant decrease in percent changes of % fat in the non-obese EX groups at 6 (-3.4%) and 12 months (-0.1%). The obese EX group had the highest decrease in % fat (-4.6%) and the exercise effect was almost significant ($p = 0.067$) at 12 months. In the obese, exercise training decreased significantly the levels of E_2 (-20.5%) at 6 months. Although percent changes in E1 were –18.3% and –20.5% at 6 and 12 months, respectively, there were not significant ($p = 0.075$ and $p = 0.34$, respectively). Serum levels of GH, IGF-I, cortisol and A-4 did not change significantly in response to exercise training.

Conclusions

In the first manuscript, it was concluded that women on HRT for short duration (2.3 ± 0.8 years) are not different in fat mass and fat distribution from no HRT

postmenopausal women. These data also do not support any effect of HRT on LST. Thus, the increased LST of the legs observed in obese women on HRT was likely due to obesity and not to HRT. HRT is associated with increased serum resting levels of E_1, E_2, GH, and cortisol, with decreased levels of IGF-I, but not with levels of A-4. In our sample, HRT, but not age, affected these hormone concentrations and we speculate that HRT has a positive effect on cortisol and GH levels in this population. Obesity (> 40% fat) is positively related to circulating levels of estrogens and negatively related to circulating levels of GH and IGF-I. In obese postmenopausal women on HRT with low serum levels of GH, the levels of IGF-I were lower than the no HRT group.

In the second manuscript, we concluded that no significant difference exist between women on HRT and women no HRT on gains in LST after one year of exercise training, as HRT plus exercise had similar increases in LST in the arms, whereas exercise alone also improved LST in total body, legs and trunk. The HRT group did not show a fat mass loss, whereas the exercise training alone, but not in combination with HRT, was effective in % fat reduction. In addition, considering obesity, the results indicate that exercise training is effective in increasing LST of the arms and preventing the decrease in LST of the legs observed in obese postmenopausal women. Changes in resting levels of anabolic and catabolic hormones are not modified by exercise training, and their chronic elevation may not be necessary for the positive adaptations in body composition observed in postmenopausal women.

Limitations

GH levels determination required 24 hrs measurement and changes in its levels, if any, may not be evident because of the pulsatile nature of the secretion, and in this study GH levels were assessed from single serum samples.

Because greater than normal protein intakes favor the hypertrophic process in the muscle, this factor may be important to control for in future resistance training studies in older people. It has been found that a slightly greater protein intake is required in athletes than in sedentary individuals to maintain the lean body mass (Tarnopolsky et al., 1988). The potential effect of protein intake on muscle mass was not considered in our study.

Originally, the study was designed to compare the effects of HRT and exercise training on body composition without considering differences in obesity levels. To compare the difference in body composition and hormone levels between obese and non-obese women taking and not taking HRT a similar number of subjects must be in all the groups.

Finally, different HRT components, doses and route of administration were used in this study. When administered in the usual dose, oral and transdermal HRT have different effects on IGF-I levels, although when similar levels of estrogen are reached in plasma, both routes produce similar IGF-I levels. Addition of progestin and testosterone to estrogen therapy produces different hormone levels and changes in body composition.

Body Composition, Steroid Hormones and Growth Hormone in Postmenopausal Women on Hormone Replacement Therapy.

Introduction

Menopause is associated with accelerated loss of bone and muscle mass (sarcopenia), two important factors in the development of osteoporosis and the increased risk of falls and prevalence of bone fractures in the elderly. Although obesity in postmenopausal women has been suggested to exert a protective effect for osteoporosis since it is associated with higher bone density and lean soft tissue (LST) mass and estrogen levels (Albala et al., 1996), excessive fat mass and predominant trunk fat distribution (android) are associated with increased levels of cardiovascular risk factors.

Menopause is accompanied by cessation of estrogen production by the ovaries. Ovarian and adrenal gland production of androstenedione (A-4) and testosterone (T) also decreases after menopause (Ushiroyama and Sugimoto, 1995; Hartman et al., 1997), although it has been reported that A-4 and T remain relatively stable up to 76 months after cessation of menses (Longcope et al., 1986). Through peripheral conversion, androgens contribute to estrogen levels in the postmenopausal years such that at the time of menopause, estrone (E_1) derived from the aromatization of A-4 in adipose and muscle tissue is the principal circulating estrogen (Siiteri et al. 1973; Pasquali & Casimirri, 1993; Rannevik et al, 1986; Matsumine et al., 1986).

Besides the decline in estrogen levels, menopause is also associated with a decline in circulating levels of growth hormone (GH) and insulin-like growth factor I (IGF-I) (Friend et al., 1996; Ho & Weissberger, 1990). This decline in serum GH and IGF-I

levels has been associated with estrogen deficiency since premenopausal women have greater levels than postmenopausal women (Rudman et al., 1981; Ho et al., 1987). In contrast, serum levels of cortisol undergo relatively unchanged (Crilly et al., 1981; Parker, 1991).

Aging- and menopause-related changes in circulating hormone levels have been associated with alterations in soft tissue composition in women. Early postmenopausal women experience an increase in abdominal fat that appears to be more related to menopause than to age (Svedensen et al., 1995; Tremollieres et al., 1996). This android fat distribution seems to be associated with an hyperandrogenic state (Evans et al., 1983:). The decline in serum estrogens, GH and IGF-I levels that accompanies menopause has been associated with the increment in adipose tissue mass, specially with android fat distribution, and the decrement in muscle mass (Heiss et al., 1995; Roubenoff et al., 1998; Rudman et al., 1985, Marin et al., 1993; Rasmussen et al., 1994).

Furthermore, obesity is also associated with alterations in circulating levels and changes in secretion and clearance patterns of several hormones including increased levels of estrogens, androgens and cortisol, and decreased GH and IGF-I (Albala et al., 1996; Seidell et al., 1990; Parker, 1991; Williams et al, 1984; Marin et al., 1993; Pasquali & Casimirri, 1993 Rasmussen et al., 1994). Although an inverse association of reduced GH levels has been associated with total fat mass and % body fat in older men and women (Rahim et al., 1998; Ruobenoff et al., 1998), this association is also observed for abdominal adiposity in near normal weight subjects (Vahl et al., 1996). With more

adipose tissue, aromatization of A-4 to E_1 increases 12-15% (Kissebah et al., 1989), thus altering the estrogen-to androgen ratio (Azziz, 1989).

Hormone replacement therapy (HRT) has demonstrated to be effective in decreasing the risk of osteoporosis, coronary artery disease, and the symptoms associated with menopause. HRT in the form of either oral estrogen alone or combined with progestin increases estrogen and GH, decreases IGF-I (Friend et al., 1996; Andersson et al., 1997; Moe et al., 1998; Dawson-Hughes et al., 1986; Frohlander & von Shoultz, 1988), but has no influence in androgen levels (Stomati et al., 1996). Estrogen administration to postmenopausal women seems to prevent the effects of estrogen deficiency on body fat distribution (Haarbo et al., 1991; Gambacciani et al, 1997). Although HRT has proved to increase circulating GH and to prevent the android distribution of body fat, it may not have an effect on lean tissue (Andersson et al., 1997; Heiss et al., 1995). These results have demonstrated that increased GH by HRT may have a lipolytic effect, but it fails to increase LST in postmenopausal women as demonstrated in GH-deficient adults and elderly women with GH administration (Sartori & Narici, 1994; Hansen et al., 1995; Thompson et al., 1995).

Cortisol response by the adrenals to HRT is controversial with contradictory results reported in the literature. An increase (Lobo et al., 1982; Tazuke et al., 1992; Burleson et al., 1998), no change (Abraham & Moroulis, 1974), and a decrease in cortisol levels (Cagnacci et al., 1997) have been reported in postmenopausal women on HRT.

There is a need for more information about the association of lean tissue mass, fat mass, fat distribution and HRT with hormonal levels modified by menopause and obesity

in postmenopausal women. Thus, the aims of this study were to test the hypothesis that the variations in body composition in postmenopausal women are correlated with levels of serum hormones and that these relationships are partially modified by HRT and obesity.

Methods

Participants

The eighty-four women in this study were recruited through local sources by flyers and newspaper advertisements. The eligibility criteria were: age 40-66 yr, at least 1 y of natural or surgical menopause (range 1-13 y), and free of chronic diseases associated with bone and calcium metabolism and muscle function. All women had normal medical history, physical exam and stress testing. None of the subjects had any history of regular exercise training or were on medication known to affect body composition and muscle strength.

Women who were either using HRT (2.3 ± 0.8 yr, n = 38) or not using HRT (no HRT, n = 46) were recruited into the study. Of the 38 women on HRT, 10 were on unopposed oral estrogen [Conjugated equine estrogens (CEE; n=8), micronized E2 (n=2)], 23 were on oral estrogen [CEE (n=18), micronized E_2 (n=3), and estropipate (n=2)] plus progestin, 2 were on oral micronized E_2 plus testosterone, and 3 were on transdermal estrogen plus progestin. Sixty one women had undergone natural menopause and twenty three had undergone surgical menopause [hysterectomy with unilateral (n=3) or bilateral (n=10) oophorectomy]. All subjects signed a written informed consent that

was approved by the Human subjects Institutional Review Board of the University of Arizona.

Anthropometry

Standing height was measured in duplicate to the nearest 1.0 millimeter using a wall-mounted stadiometer. Body weight was obtained in duplicate to the nearest 0.1 kilogram (kg) using an Accu-weigh Model 150 TK/A-58 beam scale (Metro Equipment Corp., Sunnyvale, CA). Body mass index (BMI) was calculated as the weight in kg divided by the square of standing height in meters. Waist and hip circumferences were measured in duplicate to the nearest 1.0 millimeter using a flexible steel tape. The average of two measurements was used to obtain the waist-to-hip ratio (WHR).

Body Composition

Whole body soft tissue composition was estimated from dual-energy x-ray absorptiometry (DXA) using a Lunar DPX (Lunar Radiation, Madison, WI) with the manufacturer's software version 1.3Y. Regional estimates of arms, legs and trunk mass and composition were obtained from the total body scans. Lean tissue mass was calculated as soft tissue mass minus fat mass. Percent fat (%) was calculated by dividing fat tissue (g) by the sum of total soft tissue (bone-free lean tissue plus fat tissue, g) and total bone mineral content (BMC, g) multiplied by 100. The precision of body composition, expressed as a coefficient of variation (CV), was determined from repeat scans (n = 2) in all subjects. The whole sample CV for fat and lean tissue mass were 3.3 and 2.2% (total body), 6.2 and 3.5% (arms), 4.7 and 2.9% (legs), and 4.7 and 2.9% (trunk), respectively.

Hormones

The venous blood samples were obtained between 0600 and 0900 h, after 12 h fast, to determine estrone (E_1), estradiol (E_2), growth hormone (GH), insulin-like growth factor I (IGF-I), cortisol and androstenedione (A-4). Whole blood samples were centrifuged at 3000 rpm for 15 minutes after being allowed to clot for 30 to 120 minutes. The serum was stored at - 80° C until analyses were completed. Levels of all hormones were assessed in duplicate by radioimmunoassay (RIA) and immunoradiometric assay (IRMA). Duplicates that differed by more than 10% were reassayed. Also, subjects from each of the study groups were included in each assay.

E_1, E_2, and A-4 were assayed using RIA kits from Diagnostic Systems Laboratories (Webster, TX). E_1 and E_2 levels were determined using a double antibody technique. The intra- and interassay CVs were 8.7 and 3.3% for E_1. The intra- and interassay CVs were 6.9 and 3.1% for E_2. A-4 levels were determined using a coated tube RIA kit. The intra- and interassay CVs were 5.6 and 6.5 % for A-4.

Serum levels of IGF-I were determined using IRMA kits from Diagnostic Systems Laboratories (Webster, TX). The intra- and interassay CVs were 0.03 and 4.7%, respectively. Determination of hormone levels for the E_1, E_2, A-4 and IGF-I were accomplished with the use of Immunofit EIA/RIA gamma counter software v4.0 (Beckman Instruments, Fullerton, CA).

Serum GH levels were measured by the avidin coated beads with two antibodies kit (Nichols Institute Diagnostic, San Juan Capistrano, CA). Due to low levels of GH in postmenopausal women, double of the sample amount (200uL) was used to increase the

assay sensitivity. The intra- and interassay CVs were 4.4 and 3.6% for GH. Serum cortisol levels were determined using a coated-tube assay from ICN Pharmaceuticals (Costa Mesa, CA). The intra- and interassay CVs were 8.9 and 5.7 %, respectively for cortisol. Determination of GH and cortisol levels was accomplished with the use of a gamma counter Micromedic 4/200 plus (Micromedic Systems, Huntsville, AL) and isodata software (ICN Biomedicals, Costa Mesa, CA).

Statistical Analyses

All values are expressed as means ± standard deviations. Independent t-tests were used to determine differences in subject characteristics, body composition and hormone levels between subjects on HRT and those no HRT.

To determine whether associations among soft tissue (fat and lean) composition and hormone concentrations were influenced by obesity, analyses of data from non-obese [no HRT (n=27) and HRT (n=23)] and obese [no HRT (n=19) and HRT (n=5)] women were compared. Obesity was defined as % fat ≥ 40% determined by DXA. Independent t-tests were used to determine differences in subject characteristics and body composition between non-obese (n=50) and obese (n=34) subjects. In addition, due to the greater contribution of the ovaries to A-4 and consequently to E_1 levels in postmenopausal women, we compared data from women with natural [no HRT (n =37) and HRT (n =24)] and surgical [no HRT (n =9) and HRT (n = 4)] menopause. Analysis of variance (ANOVA) was used to compare subject characteristics, body composition and hormone levels grouped by obesity and HRT use, and also for cause of menopause and HRT use. Tukey post-hoc tests were used when main effects were significant.

Multiple regression and correlation analyses were used to asses the influence of age, years postmenopause, HRT and total body LST on predicting hormone levels. All these independent variables were used with either % fat or regional fat mass and fat distribution in separate analyses. Type I error was set at 0.05.

Results

Subject descriptive statistics for women on HRT and no HRT are given in Table 3. There were significant differences between the HRT and no HRT groups in age and years postmenopause. Women on HRT were 3.3 years younger and were menopausal for 1.4 fewer years than women no HRT. There were no significant differences between the HRT and the no HRT groups in body weight, height, body mass index (BMI), WHR and all body composition variables assessed by DXA at baseline.

Hormone levels for the HRT and no HRT groups are shown in Table 4. There were significant differences between the HRT and the no HRT groups in E_1, E_2, GH and cortisol levels. As expected, mean serum values of E_1 and E_2 were significantly higher in women on HRT compared with women not on HRT. As expected, mean serum GH and cortisol levels were 46.3% and 9.7% higher in the HRT group, respectively. There were no significant differences in A-4 concentrations between the groups.

Baseline characteristics and body composition for the non-obese and obese groups are presented in Table 5. The obese group had significantly greater values in weight, BMI, LST mass (total body, arms, legs, and trunk), fat mass (total body, arms, legs, and trunk), leg LST/ fat ratio and % body fat than the non-obese group.

Table 3. Subjects characterisistics and body composition of postmenopausal women on and not on HRT.

	No HRT n=46		HRT n=38		
Variable	mean	sd	mean	sd	p
Age (yr)	57.6	4.4	54.3	4.5 *	0.00
Yr pm (yr)	6.3	2.8	4.9	2.5 *	0.02
Weight (kg)	68.5	10.6	69.4	11.5	0.71
Height (cm)	163.0	6.0	163.0	8.0	0.85
BMI (kg/m^2)	25.8	3.7	25.9	4.0	0.82
Waist/Hip ratio	0.79	0.01	0.78	0.06	0.70
TB Fat (kg)	26.9	7.8	27.0	8.6	0.96
Arm Fat (kg)	2.4	0.9	2.4	0.9	0.70
Leg Fat (kg)	10.6	3.1	10.7	3.3	0.94
Trunk Fat (kg)	12.9	4.2	12.9	4.7	0.98
Fat (%)	38.6	5.9	38.0	6.8	0.66
TB LST (kg)	38.6	4.5	39.2	4.5	0.52
Arm LST (kg)	3.8	0.7	3.8	0.7	0.97
Leg LST (kg)	12.5	1.5	12.7	1.7	0.62
Trunk LST (kg)	19.4	2.4	19.8	2.3	0.40
Trunk/Leg Fat	1.26	0.38	1.21	0.30	0.60

* $P < 0.05$.

Abbreviations: HRT= hormone replacement therapy; No HRT= not on HRT; sd= standard deviation; yr=years; Yr pm= years postmenopause; kg= kilograms; cm= centimeters; BMI= body mass index; TB= total body; LST= lean soft tissue.

Table 4. Hormonal levels of postmenopausal women on HRT and no HRT.

	No HRT n=46		HRT n=38		
Hormone	mean	sd	mean	sd	p
Estrone (pg/mL)	17.9	10.5	142.1	76.5 *	0.00
Estradiol (pg/mL)	10.9	8.6	61.1	28.7 *	0.00
GH (ng/mL)	0.73	0.62	1.36	1.24 *	0.00
Cortisol (ug/dL)	22.0	7.5	27.4	8.4 *	0.00
A-4 (ng/mL)	1.72	0.63	1.70	0.66	0.86
IGF-I (ng/mL)	144.7	43.7	118.6	46.3 *	0.01

* $P < 0.05$.
Abbreviations: HRT= hormone replacement therapy; No HRT= not on HRT; sd= standard deviation; GH= growth hormone; A-4= androstenedione.

Table 5. Baseline characteristics and body composition in non-obese and obese postmenopausal women.

	GROUPS			
	Non-obese (< 40% fat) n= 50		Obese (> 40% fat) n= 34	
Variable	Mean	SD	Mean	SD
Age (yr)	56.3	5.1	55.9	4.2
Postmenopause (yr)	5.9	2.8	5.4	2.7
Height (m)	1.62	0.07	1.63	0.06
Weight (kg)	62.7	8.2 *	78.1	7.6
BMI (kg/m^2)	23.5	2.5 *	29.2	2.8
Waist/Hip (ratio)	0.77	0.06 *	0.82	0.07
LST (kg)				
Total Body	38.0	4.9 *	40.1	3.5
Arms	3.7	0.7 *	4.0	0.6
Legs	12.2	1.7 *	13.2	1.2
Trunk	19.2	2.5 *	20.2	2.0
Fat Mass (kg)				
Total body	21.5	4.3 *	34.9	5.2
Arms	1.8	0.6 *	3.2	0.7
Legs	8.8	2.0 *	13.3	2.6
Trunk	10.0	2.5 *	17.1	3.0
Leg LST/Fat (ratio)	1.47	0.48 *	1.02	0.19
Trunk/Leg Fat (ratio)	1.2	0.4	1.3	0.3
Fat (%)	34.2	4.0 *	44.5	3.3

Abbreviations: BMI= Body Mass Index.

* Significant difference between non-obese and obese groups ($p < 0.05$).

Characteristics and hormone levels in non-obese and obese women on HRT and no HRT are presented in Table 6. The non-obese groups had lower trunk and leg fat mass than the obese groups. The obese women no HRT had significantly higher WHR than both the non-obese on HRT and non-obese no HRT. There was no significant difference in WHR between obese women on HRT and the other groups. In the women taking HRT, the obese groups had significant higher levels of E_1, E_2, and lower levels of GH than the non-obese groups. IGF-I levels were significantly lower in the obese women on HRT compared to both non-obese on HRT and no HRT. The non-obese on HRT group had significantly higher levels of cortisol compared to the non-obese no HRT group.

Hormone levels in women with natural and surgical menopause are shown in Table 7. The majority of the women in this study had undergone natural menopause (NM, n= 61) and 37 of them were no HRT whereas 24 were on HRT. Whereas in women that had undergone surgical menopause (n=23) 14 were on HRT and 9 were no HRT. There was no significant difference in E_1 and A-4 levels between the NM and the SM groups no on HRT.

Table 6. Characteristics and hormone levels in non-obese and obese postmenopausal women on HRT and no HRT.

	GROUPS							
	NON-OBESE*				OBESE**			
	No HRT n=27		HRT n=23		No HRT n=19		HRT n=15	
	mean	sd	mean	sd	mean	sd	mean	sd
Trunk fat (kg)	10.1	1.9 [b,c]	9.9	3.1 [d,e]	16.8	3.6	17.5	2.1
Leg fat (kg)	8.8	2.1 [b,c]	8.8	2.1 [d,e]	13.2	2.5	13.5	2.7
Trunk/Leg fat (ratio)	1.2	0.4	1.1	0.3	1.3	0.3	1.3	0.2
Waist/Hip (ratio)	0.77	0.06 [b]	0.78	0.06 [d]	0.84	0.09	0.81	0.04
Estrone (pg/mL)	15.6	6.6 [a,c]	124.6	75.0 [d,e]	21.2	13.9 [f]	168.9	73.1
Estradiol (pg/mL)	9.2	6.0 [a,c]	50.0	19.4 [d,e]	13.4	11.1 [f]	78.2	32.8
GH (ng/dL)	0.95	0.62 [a]	1.78	1.38 [d,e]	0.43	0.49	0.71	0.57
IGF-I (ng/mL)	153.0	48.2 [c]	134.8	48.5 [e]	132.2	34.0	93.8	29.7
Cortisol (ug/dL)	21.9	7.4 [a]	28.5	8.4	22.2	7.9	25.7	8.3
A-4 (ng/mL)	1.64	0.58	1.78	0.69	1.84	0.69	1.57	0.64

* Non-obese (< 40% fat); ** obese (> 40% fat)

[a] Non-obese No HRT vs Non-obese HRT; [b] Non-obese No HRT vs Obese No HRT; [c] Non-obese No HRT vs Obese HRT;
[d] Non-obese HRT vs Obese No HRT; [e] Non-obese HRT vs Obese HRT; [f] Obese No HRT vs Obese HRT. $P < 0.05$.

Table 7. Hormonal levels in natural and surgical postmenopausal women.

	GROUPS							
	NATURAL MENOPAUSE				SURGICAL MENOPAUSE			
	No HRT n=37		HRT n=24		No HRT n=9		HRT n=14	
Variables	mean	sd	mean	sd	mean	sd	mean	sd
Estrone (pg/mL)	18.3	11.5 [a,b]	144.9	75.8 [c]	16.4	5.2 [d]	137.2	80.2
Estradiol (pg/mL)	10.7	9.0 [a,b]	57.7	24.7 [c]	11.6	7.3 [d]	67.0	34.7
GH (ng/dL)	0.73	0.58 [a]	1.40	1.23	0.74	0.79	1.28	1.28
Cortisol (ug/dL)	21.7	7.3 [a]	27.9	7.3	23.5	8.6	26.6	10.2
A-4 (ng/mL)	1.74	0.63	1.70	0.77	1.65	0.65	1.69	0.43

GH= Growth Hormone; A-4= Androstenedione. Natural Menopause= NM; Surgical Menopause=SM.

[a] NM No HRT vs NM HRT; [b] NM No HRT v SM HRT; [c] NM HRT v SM No HRT; [d] SM No HRT v SM HRT ($p < 0.05$).

Pearson's correlation coefficients between hormone levels and HRT use, obesity and soft tissue are shown in Table 8. E_2 was significantly correlated with HRT (r=0.78) and obesity (r=0.21) and almost significantly with trunk fat mass (r=0.21; p=0.05). Cortisol was significantly correlated with HRT (r=0.32). GH was positively correlated with HRT (r=0.31) but negatively correlated with obesity, % fat, trunk and leg fat mass (r=-0.25 to –0.39). IGF-I was negatively correlated with HRT, obesity, WHR, trunk/leg fat mass ratio, % fat and trunk fat mass (r=-0.23 to -0.35).

The correlation coefficients were examined by HRT use to differentiate the association between these variables in the HRT and no HRT groups (Table 9). Significant correlations between A-4 and cortisol, cortisol and E_1, E_1 and E_2, GH and % fat, A-4 and age, and age and years postmenopause were found for both groups. In the no HRT group, A-4 was significantly correlated with E_1 and E_2; also, E_1 was significantly correlated with % fat. On the other hand, except for a significant negative correlation between IGF-I and % fat in the HRT group, there were no significant differences between both groups.

Multiple regression analyses with age, years postmenopause, HRT status, total body LST and % fat as independent variables to predict hormone status are presented in Table 10. HRT status was positively related to E_1, E_2, GH and cortisol levels but negatively related with IGF-I levels. Substituting % fat by arm, leg and trunk fat mass, the association between HRT and E_1, E_2, GH, IGF-I and cortisol were not different than in the first analysis (Table 11). In addition, a positive association was found between trunk fat mass and E_2, and A-4, whereas arm fat was negatively related with A-4. LST in total body was positively associated with IGF-I.

Table 8. Correlation coefficients for hormones and body composition variables.

Variable	A-4	Cortisol	E1	E2	GH	IGF-I	% Fat	Age, yr	PM, yr	Trk/Leg	Trk Fat	Leg Fat	WHR	TBLST	HRT	Obesity
A-4	1.00															
Cortisol	0.46*	1.00														
E1	0.12	0.41*	1.00													
E2	0.06	0.29*	0.89*	1.00												
GH	0.02	0.05	0.08	0.06	1.00											
IGF-I	0.19	-0.03	-0.17	-0.27*	0.13	1.00										
% Fat	0.02	-0.04	-0.03	0.10	-0.34*	-0.34*	1.00									
Age (yr)	-0.02	-0.16	-0.26*	-0.31*	-0.10	-0.01	0.03	1.00								
PM (yr)	-0.07	-0.04	-0.21	-0.30*	-0.05	0.10	0.01	0.62*	1.00							
Trk/Leg fat	0.11	-0.06	0.02	0.09	-0.18	-0.28*	0.11	0.19	0.01	1.00						
Trk fat	0.11	0.01	0.06	0.21*	-0.39*	-0.35*	0.88*	0.06	0.00	0.44*	1.00					
Leg Fat	0.03	-0.01	0.00	0.11	-0.25*	-0.19	0.85*	-0.06	0.01	-0.25*	0.72*	1.00				
WHR	0.15	0.02	0.02	0.13	-0.17	-0.23*	0.34*	0.11	0.02	0.68*	0.62*	0.14	1.00			
TBLST	0.11	-0.04	0.02	0.18	-0.07	-0.01	0.11	-0.03	-0.01	0.29*	0.45*	0.31*	0.48*	1.00		
HRT	-0.02	0.32*	0.77*	0.78*	0.31*	-0.28*	-0.05	-0.35*	-0.26*	-0.06	0.00	0.01	-0.03	0.07	1.00	
Obesity	0.01	-0.08	0.13	0.21*	-0.39*	-0.32*	0.81*	-0.03	-0.08	0.19	0.79*	0.71*	0.39*	0.23*	-0.02	1.00

* Significant correlation ($p < 0.05$). A-4= androstenedione; E1= estrone; E2= estradiol; GH= growth hormone; IGF-I= insuline-like growth factor-I; PM= postmenopause; Trk= trunk; WHR= waist to hip ratio;TBLST= lean soft tissue in total body.

Table 9. Correlations between hormones, age and percent fat in postmenopausal women (n=84) considering the effect of HRT.

Variables	No HRT (n=46)	HRT (n=38)
A-4 and E_1	0.48 *	0.24
A-4 and E_2	0.40 *	0.04
A-4 and age	-0.30 *	0.29 **
E_1 and % Fat	0.36 *	-0.03
IGF-I and % Fat	-0.17	-0.55 *

* Significant correlation ($p < 0.05$).
** $p = 0.08$
Abbreviations: HRT= Hormone Replacement Therapy; No HRT= Not on HRT; A-4= Androstenedione; E_1 = Estrone; E_2= Estradiol; GH= Growth Hormone; IGF-I= Insulin-Like Growth Factor I.

Table 10. Multiple linear regression for prediction of hormone levels by independent variables in postmenopausal women on HRT and no HRT.

Independent variables	Dependent variables					
	E_1	E_2	GH	IGF-I	F	A-4
Age, yr	0.25	0.22	-0.01	-2.01	-0.23	0.01
Yr-PM, yr	-0.5	-1.47	0.02	2.47	0.37	-0.02
HRT	62.37 *	24.35 *	0.31 **	-15.47 **	2.62 **	-0.03
% fat	0.18	0.61	-0.05 **	-2.60 *	-0.03	0.00
LST, kg	-0.001	0.001	0.000	0.001	0.000	0.000
R^2	0.59	0.65	0.21	0.23	0.12	0.02
SEE	53.17	19.72	0.92	42.23	8.06	0.65

* $P < 0.05$; ** $P < 0.01$; * $P < 0.001$

Abbreviations: F= cortisol; GH= growth hormone; IGF-I= insulin-like growth factor I; A-4= androstenedione; E1= estrone; E2= estradiol; Yr-PM= years postmenopause; HRT= hormone replacement therapy (1= use of HRT, -1 not use of HRT); and LST= lean soft tissue in total body; SEE= standar error of estimate.

Table 11. Multiple linear regression for prediction of hormone levels by independent variables in postmenopausal women on HRT and no HRT.

Independent variables	Dependent variables					
	E_1	E_2	GH	IGF-I	F	A-4
Age, yr	-0.31	-0.03	0.005	-1.5	-0.29	-0.001
Yr-PM, yr	0.15	-1.18	0.02	2.2	0.47	-0.01
HRT	62.29 "	24.19 "	0.34 **	-14.3 **	2.71 **	-0.01
Arm fat, kg	-0.005	-0.002	-0.0002	-0.02	-0.002	-0.0006 **
Leg fat, kg	-0.003	-0.0008	0.00003	0.003	-0.0001	0.00001
Trunk fat, kg	0.004	0.002 *	-0.0001	-0.003	0.0006	0.0001 **
LST, kg	-0.001	0.0003	0.00003	0.003 *	-0.0001	0.00003
R^2	0.61	0.67	0.27	0.29	0.13	0.13
SEE	52.99	19.42	0.89	41.07	8.09	0.62

* $P < 0.05$; ** $P < 0.01$; " $P < 0.001$

Abbreviations: F= cortisol; GH= growth hormone; IGF-I= insulin-like growth factor I; A-4= androstenedione; E1= estrone; E2= estradiol; Yr-PM= years postmenopause; HRT= hormone replacement therapy (1= use of HRT, -1 not use of HRT); and LST= lean soft tissue in total body; SEE= standar error of estimate.

Discussion

We found no differences between the HRT and the no HRT groups in weight, BMI, WHR, total and regional fat mass, % fat and fat distribution. Women on HRT were 3.3 years of age younger and with a mean of 1.4 years since menopause less than women no HRT. Women on HRT were included only if they were using estrogens for 1 to no more than 3.9 years which resulted in their being early postmenopausal. Our findings were consistent with a previous study by Kritz-Silverstein et al. (1996), who found no differences in fat mass and WHR between postmenopausal women on unopposed CEE compared to women not on HRT after 15 years of follow-up. In contrast, Hanggi et al. (1998) demonstrated that DXA-determined body fat distribution did not change significantly in postmenopausal women after using oral E_2-P for 2 years, whereas the increase in fat mass and central fat distribution was not prevented in women no HRT. In another prospective study, Reubinoff et al. (1995) observed that one year of oral CEE 0.625 mg and medroxyprogesterone 2.5 mg did not prevent the increase in body weight, BMI and % body fat in early postmenopausal; however, HRT attenuated the increase in abdominal fat observed in women not on HRT. Differences in age and % body fat may contribute for these conflicting results when the effects of HRT on fat mass and fat distribution are associated to HRT.

In our study, although the majority of HRT users were on combined CEE with progestin, there was not a significant difference in body fat distribution, determined by trunk/leg fat ratio and WHR between women on HRT and no HRT. However, when stratifying the subject groups by obesity, it was found that the obese had a higher WHR

than the non-obese with no difference in trunk/leg fat ratio. Considering HRT use and obesity, WHR was higher in the obese no HRT compared to both non-obese groups (HRT and no HRT) whereas no difference was observed between both obese women groups, on HRT and no HRT. However, the trunk fat and the leg fat mass were higher in the obese groups than in the non-obese groups regardless of HRT. Gambacciani et al. (1997) reported that HRT with oral E_2-P combination for 12 months can prevent gains in trunk fat but not in leg in postmenopausal women with lower fat mass (16.3 %), BMI (5.8%) and greater time since menopause (7.2 years) than our obese subjects. In our data, using a pooled sample of obese and non-obese with and without HRT, no significant correlation was found between HRT and % fat, trunk fat mass, leg fat mass, trunk/leg fat ratio and WHR. These results suggest that HRT is more effective in attenuating the increase in WHR but it can not prevent the accumulation of fat neither in the trunk nor in the legs in obese postmenopausal.

In the present study, we found no difference in total body and regional LST between the HRT and no HRT groups. When women were classified as non-obese and obese, the obese women had higher fat and LST mass in total body, arms, legs, and trunk. It is known that postmenopausal women with higher fat mass also have higher muscle mass (Roubenoff et al., 1998). Since approximately 25% of the weight gain is estimated to be LST (Forbes, 1987), higher LST in obese women, regardless of HRT, suggests that obesity and not HRT is the factor that accounts for this difference. Results of the present study in non-obese postmenopausal women are in accord with those from Gambacciani et al. (1997), who found no significant changes in DXA-determined total and regional LST

in either controls or in women on estrogen-progestin combination for one year. Conversely, in a recent study Hanggi et al. (1998) found that non-obese controls and oral E_2-P treated postmenopausal women had a decrease in trunk LST, but not in the arm and legs using DXA. Moreover, transdermal E_2-P HRT also did not have a significant effect in LST of the extremities and the trunk. Because the majority of the muscle mass is in the extremities (Heymsfield et al., 1990; Wang et al., 1996), these results suggest that the decrease of LST associated with menopause appears to be prevented but not improved by either oral or transdermal HRT.

At rest, circulating levels of E_1, E_2 and A-4 are similar than those usually reported for postmenopausal women on HRT and no HRT (Tazuke et al., 1992; Ushiroyama & Sugimoto, 1995; Andersson et al., 1997; Kostoglou-Athanassiou et al., 1997). As expected, women on HRT had higher serum E_1 and E_2 than women no HRT. Conversions of androgens to estrogens in peripheral tissues are greater in postmenopausal women as compared to those levels observed in premenopausal women. It has been shown that A-4 is converted to E_1, and E_1 can be aromatized to E_2 in adipose tissue (Longcope, 1998). We found that these conversions are important in the no HRT group, as evidenced by the significant correlations between A-4 with E_1 and E_2. However, estrogen levels in postmenopausal women on HRT depend on exogenous estrogen and not on androgen conversion. Although oral HRT with CEE and E_2 results in higher levels of E_1 than E_2 (Bellantoni et al., 1996; Tazuke et al., 1992), E_1 levels may depend on factors other than adiposity. One possible factor could be that aromatization of A-4 to E_1 is slightly decreased in humans after adrenocorticotropic hormone (ACTH) and/ or glucocorticoids

administration (Longcope, 1987). The failure to find a correlation between either % fat or regional fat mass and E_1 levels in our women on HRT suggests that peripheral conversion of A-4 to E_1 may be decreased in postmenopausal women with high cortisol levels.

In the present study, estrogen levels were higher in obese women, although significantly different only in women on HRT. A significant correlation has been reported between degree of obesity and E_1 and to a lesser extent E_2 suggesting a possible role of adipose tissue in the conversion of androgens to estrogens in postmenopausal women (Vermeulen & Verdonck, 1978). In the present study, no significant correlations were found between E_1 or E_2 and trunk/leg fat, leg fat mass, % fat, WHR, and trunk fat mass. However, using multiple regression, both E_1 and E_2 were positively associated with HRT whereas only E_2 was positively associated with trunk fat mass. However, an almost significant ($p=0.052$) correlation between E_2 trunk and fat mass together with a significant correlation with obesity further suggests that trunk fat mass may play an important role in the conversion of sex hormones to E_2 observed with increasing adiposity in postmenopausal women on HRT.

In the present study, greater serum GH levels accompanied increased estrogen levels in women on HRT. Our finding of positive associations between HRT and GH levels are in agreement with previous studies in which serum GH concentrations were increased in postmenopausal women on HRT (Friend et al., 1996; Andersson et al., 1997; Moe et al., 1998; Frohlander & von Schoultz, 1988). HRT increases GH secretion through reduced negative feedback on the pituitary while decreasing serum IGF-I levels (Dawson-Hughes et al., 1986; Friend et al., 1996; Andersson et al., 1997: Mercuri et al.,

1993; Frohlander & von Schoultz, 1988; Helle et al., 1996). This increase in GH levels was observed in non-obese women on HRT who were younger than non-obese women no HRT. Recently, Bellantoni et al. (1996) reported that oral HRT increased GH release in younger and older postmenopausal women, although significantly so only in the younger women compared to their own baseline value with no difference between the groups.

The non-obese women no HRT had the greatest levels of IGF-I and were significantly different compared to the obese on HRT but not to the non-obese on HRT women. Only 3 of the 38 women on HRT used the transdermal administration and all were non-obese. HRT in low doses or by transdermal administration enhances IGF-I levels, while resulting in similar serum E_2 concentrations, both oral and high doses of transdermal administration decrease IGF-I levels (Cano et al., 1999; Moe et al., 1998; Friend et al., 1996). Considering the 84 women as well as those in the no HRT group, GH and IGF-I did not show significant correlations with E_2. Conversely, HRT was positively and negatively associated with GH and IGF-I, respectively.

In our study, obesity defined as 40% fat (mean BMI= 29.3 kg/m^2) had a negative effect on GH and IGF-I concentration. Obese women on HRT had lower GH and IGF-I levels than non-obese women on HRT and no HRT. Low GH and IGF-I concentrations in serum have been previously been reported in obese subjects (Rudman et al., 1981; Marin et al., 1993). GH stimulates the hepatic production of IGF-I and obesity influence both hormone levels. Low levels of IGF-I may indicate a deficient GH secretion in obese subjects that is reverted with lower fat levels (Williams et al. 1984; Marin et al., 1993). Our findings of a negative association by multiple regression analysis and correlation

coefficients between % fat and GH, and IGF-I levels confirms the association of lower GH and IGF-I levels with indices of adiposity in a population of postmenopausal obese women on HRT (Moe et al., 1998; Roubenoff et al. 1998; Rasmussen et al., 1994 and 1995; Marin et al., 1993). Furthermore, the significant correlations among these hormones and variables of fat distribution, seems to indicate that fat accumulation in the trunk has a major impact in the decrease of GH and IGF-I levels.

Although circulating IGF-I levels appear to reflect endogenous GH levels under physiological conditions, obesity in combination with HRT seems to have an additive effect on the relationship between these hormones than either factor alone. The negative relationship between GH and % fat but not with HRT, and IGF-I with HRT and % fat is thus implying a diminished GH secretion by obesity that results in decreased IGF-I that is more pronounced with HRT use. A suggested direct inhibitory effect of HRT on hepatic IGF-I synthesis in addition to lower serum GH levels might explain the decreased IGF-I levels in the obese on HRT (Dawson-Hughes et al., 1986; Friend et al., 1996: Andersson et al., 1997; Frohlander & von Schoultz., 1988).

As previously reported by several studies, we found that women on HRT had higher cortisol levels than women not on HRT. Tazuke et al. (1992) found this effect in non-obese postmenopausal women using oral CCE. The findings of a positive association, and also a significant correlation between HRT and cortisol levels confirm the known stimulatory effect of estrogen therapy either as oral contraceptive or HRT on cortisol levels in women (Tazuke et al., 1992; O'brien et al., 1993). Abraham and Maroulis (1974) have suggested that HRT may increase the effect of ACTH on the

secretion of cortisol by the adrenal cortex. It is known that HRT stimulates the steroidogenic enzymes that leads to adrenal androgens and cortisol synthesis (Lobo et al., 1982) resulting in augmented serum levels of steroid hormones in response to ACTH (Johnson et al., 1997).

Our results are in agreement with those of Grenman et al. (1986) who found that postmenopausal obese women no HRT do not differ from normal weight women in serum cortisol levels. In our study, we found that in non-obese and obese women on HRT the levels of cortisol are higher than women no HRT, but it is only significant in the non-obese on HRT who also have higher levels of GH. Our finding that both estrogens are significantly correlated with cortisol may suggest that the high cortisol levels found in non-obese women on HRT reflect a stimulatory effect of estrogens on the adrenal glands (Tazuke et al., 1992). Burleson et al. (1998) likewise found that women on HRT have higher cortisol levels with no manifestations of hypercortisolemia. Although we did not measure the levels of cortisol-binding globulin (CBG), a well known stimulatory effect on hepatic protein synthesis (CBG and sex hormone binding globulin) has been described to be associated with HRT. The binding of cortisol to CBG results in a decreased metabolic clearance rate (MCR) that increases cortisol levels (Rosenthal et al., 1969; Nolten et al., 1980).

Furthermore, the finding of comparable ACTH levels in women on HRT and no HRT suggests that the bioavailable free cortisol fraction in both groups may be similar (Burleson et al., 1998). Marin et al. (1992) reported that in response to ACTH analogues and stress, increased visceral fat is associated with elevated cortisol secretion in obese

premenopausal women. Although HRT increases cortisol secretion in obese women, there is a compensatory increment in its MCR (Parker, 1991). The hyperinsulinemia associated with obesity decreases hepatic protein synthesis (Frystyk et al., 1995) resulting in more free hormone, which consequently may increase 24-h urinary cortisol excretion that lead to low serum cortisol levels in these women (Marin et al., 1992).

Although we found that HRT had a stimulatory effect on cortisol secretion in younger non-obese postmenopausal women, A-4 levels, which are partially regulated by ACTH, were not affected. Moreover, we found similar A-4 levels in all postmenopausal women independent of age, years postmenopause or levels of obesity. Whereas adrenal androgens and cortisol secretion is determined by ACTH stimulation (Azziz et al., 1990), the aging process may alter the secretion of these hormones. A decreased androgen secretion and increased cortisol levels in response to human corticotropic releasing factor or ACTH have demonstrated the changes in the capacity of the adrenals to secrete steroid hormones in postmenopausal women (Liu et al., 1990; Akamatsu et al., 1992). Rozenberg et al. (1988) observed a fall in the levels of T and A-4 after age 50. In a longitudinal study, Rannevick et al. (1986) demonstrated a decrease in A-4 levels (16 %) at menopause with a further decrease of about 15% in the following three years. In our study, women who were less than 5 years postmenopause (n=45) did not differ in A-4 levels with women over five years postmenopause (n=38).

We did not detect significant differences in serum A-4 levels between non-obese and obese. In a recent study, Vicennati et al. (1998) observed that A-4 and T levels are similar in normal weight and obese premenopausal women. We found that obesity does

not alter A-4 levels and that this androgen is independent from ovarian secretion from 3 to 13 years postmenopause.

Because there is a dramatic fall in androgen levels after oophorectomy (Lobo et al., 1982), we subsequently analyzed women with natural and surgical menopause, finding no differences in A-4 levels. The study of Vermeulen (1976) showed that in postmenopausal women, the adrenals are the major source of A-4 and about 50% of the basal levels of T that are converted into E_1 and E_2, respectively. Our results in regards to the serum concentrations of A-4 in natural and surgical postmenopausal women are in agreement with those of Abraham and Maroulis (1974) and Castelo-Branco et al. (1993), who reported no change in its levels after HRT. The present study indicates that A-4 levels are not modified by HRT use.

In summary, the findings of the present study confirms that women on HRT for short duration (2.3 $\pm$ 0.8 years) are not different in fat mass and fat distribution than postmenopausal women not on HRT. These data also do not support any effect of HRT on lean soft tissue mass. Thus, the increased lean mass in total body, arms, legs and trunk observed in obese women was likely due to obesity and not to HRT. HRT is associated with increased serum resting levels of E_1, E_2, GH, and cortisol, and also with decreased levels of IGF-I. HRT was not associated with levels of A-4. In our sample, HRT but not age affected these hormone concentrations and we speculate that HRT has a stimulatory effect on the somatotropic and corticotropic axis in this population. Obesity (> 40% fat) is positively related to circulating levels of estrogens and negatively related to circulating

levels of GH and IGF-I. In obese postmenopausal women with low serum levels of GH, the addition of HRT produces a greater decrease in IGF-I levels.

Effects of Resistance Exercise Training and Hormone Replacement Therapy on Lean Soft Tissue and Fat Mass, Anabolic Hormone and Catabolic Hormones in Postmenopausal Women.

Introduction

Changes in body composition in normal individuals with aging include a decline in bone mass and lean soft tissue (LST), and increased fat mass (Forbes & Halloran, 1976). These changes have important implications for health and functional capacity. Sarcopenia, an excessive loss of muscle mass, may be a factor by which older persons become frail and at higher risk for falls and bone fractures. An average loss of 6 to 7% of LST, especially in the legs, has been reported in healthy men and women from 65 to 85 years of age (Baumgartner et al., 1995), and sarcopenia is an important contributor to the age-related loss in muscle strength (Frontera et al., 1991). In addition to the gain in body fat with advancing age, fat distribution changes, favoring an increase in the ratio of upper-to lower-body fat in both sexes with a concomitant increase of cardiovascular disease risk (Horber et al., 1997).

Changes in LST and fat mass are related to alterations in hormone levels and activities associated with the menopause. The gradual decline in sex hormones, growth hormone (GH) and insulin-like growth factor I (IGF-I) with aging may be related to sarcopenia. Decreasing basal levels of androgens in older people, especially in women, are related to the sex and age-dependent differences in muscle strength and the decreasing anabolic effects on LST (Hakkinen & Pakarinen, 1993). Because GH causes an increase in LST and reduction in fat mass, the age-associated decrease in GH and IGF-

I levels has been related with the modifications in body composition commonly observed in older adults (Rudman, 1985, Benbassat et al., 1997). Lower GH levels in postmenopausal women compared to premenopausal women may be due to higher estrogen levels that enhance GH secretion in premenopausal women (Frantz & Rabkin, 1965).

Whether the decrease in LST is an effect of aging or a result of estrogen deficiency is not entirely clear. Although hormone replacement therapy (HRT) is commonly recommended to prevent bone mass loss, its contribution to maintaining LST has not been clearly demonstrated (Gambacciani et al., 1997). Considering that women on HRT have higher levels of GH and that GH treatment produces increases in muscle mass in GH-deficient adults (Friend et al., 1996; Sartori & Narici, 1994), it is possible that higher levels of GH may have an anabolic effect in women on HRT. On the other hand, in the study by Taafee et al. (1995) lower-body muscle strength and lean body mass of postmenopausal women was not influenced by HRT, suggesting that estrogen deficiency is not an important factor in maintaining LST mass and function in non-exercising older women.

The age-related decrease in LST mass can be partially prevented or restored by intense exercise training. Cross-sectional studies in older athletes suggest that intense chronic training prevents the decline in LST and the increase in fat mass in women and men (Ryan et al., 1996; Klitgaard et al., 1990). Several studies have reported that resistance exercise training is an effective means to improve neuromuscular function, muscle mass and strength in young and elderly men and women (Frontera et al., 1988;

Cureton et al., 1988; Fiatarone et al., 1990; Nelson et al., 1994; Treuth et al., 1994). Previous resistance exercise training studies have shown significant increases in muscle mass and decreases in intramuscular fat in postmenopausal women not on HRT (Nelson et al., 1994; Charette et al., 1991; Pyka et al., 1994a; Ryan et al., 1995; Sipila et al., 1995). Although several prospective studies have tested the efficacy of resistance exercise training in augmenting LST in postmenopausal women not on HRT, very few training studies have been conducted combining exercise and HRT in postmenopausal women (Brown et al., 1997; Kohrt et al., 1998; Thompson et al., 1998). Brown et al. (1997) reported that HRT did not have an additive effect on the increase on leg LST in postmenopausal women aged 60-72 years after 11 months of walking, jogging and stair climbing exercise training compared to e aged 60-72 years after 11 months of walking, jogging and stair climbing exercise training compared to exercise alone. With a similar population and exercise training program, Kohrt et al. (1998) found that HRT alone and HRT plus exercise attenuated fat accumulation, especially in the abdomen.

The increase in LST mass, the decrease in fat mass and change in fat distribution in response to exercise training appears to be brought about through changes in the hormonal milieu. Acute exercise and exercise training may elicit significant changes in blood hormone levels that may be associated with positive changes in body composition (Cumming et al., 1987; Kraemer et al., 1991 and 1993; Weiss et al, 1983; Hutchinson et al., 1981; Staron et al., 1994). The majority of the studies have not shown increases in testosterone (T) levels in females following acute resistance exercise or training (Hakkinen et al., 1990; Kraemer et al., 1991, Westerlind et al., 1987). In contrast, acute

increases in response to heavy resistance exercise in androstenedione (A-4) levels have been observed in young females (Kraemer et al., 1995a; Weiss et al., 1983). Although A-4 is a less potent androgen than T, it may be important in women because A-4 is more abundant than T, and the resting and post-exercise A-4 levels are higher in women than in men (Weiss et al., 1983). Thus, a similar A-4 response to resistance exercise in both sexes coupled with higher resting levels of this androgen may contribute to muscle hypertrophy in women in response to prolonged heavy resistance training. Previous studies have found high resting levels of A-4 in young competitive female swimmers (Constantini & Warren, 1995) and athletes participating in anaerobic sports (Hutchinson et al., 1981).

The lack of exercise-induced increases in T levels in women may mean that other potent anabolic hormones such as GH and IGF-I may participate in muscle adaptations to resistance training (Kraemer et al., 1991 and 1993). Although GH levels do not increase similarly with all strength exercise protocols, resistance exercise at 10 repetition maximum (RM) leads to significant greater acute increases in GH levels in young and middle-aged men and women (Kraemer et al., 1991 and 1992; Hakkinen & Pakarinen, 1995). While the response is decreased in elderly men and women, three out of six subjects of the older group showed a significant GH response to a load of 85% of 1 RM (Pyka et al., 1992). However, no change in resting GH levels in older men and women after one year of resistance exercise training was reported (Pyka et al. 1994b). These results suggest a great individual variability and a possible significant GH response to heavy resistance exercise in some elderly individuals.

In addition to anabolic hormones, cortisol levels have been considered important in the study of the hormonal responses and adaptations to resistance exercise (Hakkinen et al., 1985 and 1994). Due to its catabolic effects, it may be especially important in postmenopausal women on HRT due to the increase in cortisol levels associated with HRT (Lobo et al., 1982). Some studies have demonstrated decreases in resting levels of cortisol during the course of 8 to 24 weeks of resistance training in young men and women (Hakkinen et al., 1985, Hetrick & Wilmore 1977; Kraemer et al., 1998) and elderly women (Hakkinen and Pakarinen, 1994). On the contrary, when the training programs have been overly stressful and inadequate recovery is allowed, the increase in cortisol could lead to an overtraining state that might compromise muscle adaptations (Hakkinen et al., 1987, 1989). Thus, chronic alterations in cortisol levels may be of importance for muscle adaptations to exercise training.

Obesity and android fat distribution have a major influence in the secretion and resting levels of estrogens, androgens, GH and IGF-I (Albala et al., 1997; Williams et al., 1984; Dawson-Hudges et al., 1986; Rudman et al., 1981; Marin et al., 1993). The obesity-related decline in the activity of the GH/IGF-I axis may be associated to the lower gains in LST observed in response to resistance training in obese women on HRT (Thompson et al., 1998).

Because women on HRT may respond to resistance exercise training differently from women not on HRT, the effects of exercise on anabolic and catabolic hormone adaptations underlying adaptations in LST and fat mass need to be investigated in both populations. Moreover, obesity may be a factor that influences negatively these

adaptations. The results of the present study will show whether prolonged resistance exercise-training program is an effective adjunct or alternative to HRT for maintaining or improving muscle mass in postmenopausal women. Thus, the purpose of this study was to determine whether an exercise training program combined with HRT results in different gains in LST, fat mass loss, and adaptations in hormone levels in non-obese and obese postmenopausal women than a similar exercise training program without HRT.

Methods

Subjects

A group of eighty four previously sedentary women aged 40-65 y, who were three to ten years postmenopausal were accepted to participate in the study. Women taking HRT (n=38) and women not taking HRT (no HRT, n=46) were randomly assigned into exercise (EX) or no exercise groups resulting in the following groups: exercise plus HRT (EX+HRT, n=16)], exercise (EX, n=20), no EX on HRT (HRT, n=22) and no EX and no HRT [(CONTROL (CONT), n=26]. All subjects were instructed to maintain their body weight over the duration of the study. The no exercising (HRT and CONT) groups were asked to maintain their physical activity and diet habits during the study. Participants were screened by medical history, physical examination and a graded treadmill exercise test with blood pressure and maximal oxygen consumption (VO_2 max) measurement. Women were excluded from the study if they had medical problems that contraindicate exercise and use of hormonal therapy other than HRT. After explanation of the study, all subjects gave written consent to participate. The protocols were approved by The University of Arizona's Human Subjects Committee.

Exercise Training

The supervised training program was conducted three times per week on alternating days and consisted of both resistance and weight-bearing aerobic exercises. The resistance exercises were leg press, squat, lateral pull down, seated row, back extension, military dumbbell press and rotary torso. Workouts consisted of 2 sets of 6-8 repetitions with 45 to 60 seconds of rest between sets for each exercise, alternating between medium 70% of 1 repetition maximum (1 RM) and high intensity (80% 1 RM) days.

Weight-bearing exercise training consisted of warm up walking (5 min), stepping with weighted vest (10 min) and walking with weight vest (10 min). Walking was combined with skipping, jogging, hopping and jumping. These activities were performed at an intensity of 50-80% of maximal heart rate. The load in the weighted vest was progressively increased from 4 to 14 kg.

Strength Testing

Strength testing was performed on all exercise subjects to assess changes in muscle strength in selected muscle groups. The dynamic muscle strength was determined for muscles of the thigh, back and shoulders using the one repetition-maximum (1 RM) test. The 1 RM was the maximal weight that could be lifted through a full range of motion using a correct form. The 1 RM strength was determined for all the exercises included in the resistance training every 6 weeks to progressively increase the resistance and maintain the training intensity throughout the study. Only 1 RM of leg press, seated row and military press at baseline 12 months and the changes from baseline at 12 months

were used in the analysis. Percent changes at 12 months in muscle strength were calculated as follow: % change = [(12 month value – baseline value) x 100] / baseline value.

Dual-Energy X-Ray Absorptiometry (DXA)

Women were scanned on medium speed using a DXA whole body scanner model DPX-L (Lunar Radiation, Madison, WI). The system software (version 1.3) divides pixels first into bone mineral (BM) and soft tissue mass. The soft tissue is then further separated into fat and lean soft tissue (LST). Total body was segmented to obtain estimates of arms, legs, and trunk LST and fat mass using the manufacturer's software. Total body percent (%) fat was calculated as follows: % fat = [fat mass / (fat mass + LST + BM)]. Each subject was scanned two times within a week at each measurement interval (baseline, 6 and 12 months) to improve the precision of DXA measurements. The technical error, expressed as coefficient of variation (CV), for total and regional fat and LST varied from 2.2 to 9.8%. The average of the two scans for various compartments was used in subsequent analyses due to the high correlations between them (r=0.94-0.99).

Serum Collection

All blood samples were obtained after a 12-hour fast. Blood draws were made 24-48 hr after the last exercise training session to avoid a possible acute effect of exercise on hormone levels. Following standard venipuncture procedures, whole blood was collected into serum vacutainer tubes and allowed to clot at room temperature for 30-120 min. After separation (15 min at 1500 rpm), serum samples for hormone analyses were kept

frozen at -80° C until assayed. Collections were at a similar time of day (between 0600 and 0900 h) for baseline, 6 and 12 months to reduce the effects of diurnal variations on hormone levels.

Hormone Analyses

Serum levels of estrone (E_1), estradiol (E_2), androstenedione (A-4), growth hormone (GH) and cortisol were determined by radioimmunoassay (RIA) methods. The E_1, E_2, and A-4 kits were obtained from Diagnostic Systems Laboratories (Webster, TX). The intra- and inter-assay CVs were 8.7 and 3.3% for E_1. The intra- and inter-assay CVs were 6.9 and 3.1% for E_2 and 5.6 and 6.5% for A-4, respectively. The GH kit was obtained from Nichols Institute Diagnostics (San Juan Capistrano, CA). The intra- and inter-assay CVs for GH were 4.4 and 3.6%, respectively. For the measurement of cortisol, kits from ICN Biomedicals (Costa Mesa, CA) were used. The intra- and inter-assay CVs for cortisol were 8.9 and 5.7%, respectively.

Serum levels of IGF-I were determined using immunoradiometric kits from Diagnostic Systems Laboratories (Webster, TX). The intra- and interassay CVs were 0.03 and 4.7%, respectively.

A dose response curve of radioactivity vs concentration was generated from the standard concentrations using Immunofit EIA/RIA software version 4.0 (Beckman Instruments, Fullerton, CA) for E_1, E_2, A-4 and IGF-I. Concentrations of the unknowns were determined from these curves. The Micromedic 4/200 plus (Micromedic Systems, Huntsville, AL) and isodata software (ICN Biomedicals, Costa Mesa, CA) were used to

derive cubic spline standard curves which enabled determination of GH and cortisol levels from measurements of antibody-hormone in the unknown samples.

Baseline, 6 and 12 month samples from each subject were processed in duplicate in the same assay to minimize inter-assay variability. Also, samples from all four study groups were analyzed in the same assay. All samples with differences between duplicates higher than 10% or with values below and above the lowest and highest standard were reassayed.

Statistical Analyses

Differences in body composition and hormone levels at baseline among EX+HRT, EX, HRT and CONT groups as well as differences among non-obese and obese (no EX and EX) subjects were assessed by one-way ANOVA. Tukey post-hoc tests were used to identify significantly different means among the groups.

Multiple regression analyses were used to determine the effects of HRT (no HRT vs HRT), the effects of exercise training for women no HRT (EX vs CONT), and the effects of exercise training for women on HRT (EX+HRT vs HRT) on body composition and hormone levels changes at 6 and 12 months. To determine the effects of exercise training in non-obese and obese subjects on body composition and hormonal changes, multiple regression analyses were also used. Percent changes at 6 and 12 months in all variables were calculated as follow: % change = [(6 or 12 month value – baseline value) x 100] / baseline value. Changes from baseline at 6 and 12 months were entered as the dependent variables and coding for HRT (HRT vs no HRT, EX vs CONT, and EX+HRT vs HRT) and for obesity [non-obese vs obese, non-obese (no EX vs EX), and obese (no

EX vs EX)], age, years postmenopause, and the baseline value as the independent variables. Baseline values as covariates were used to account for the influence of the initial value on the changes at each time point. Adjusted means and percent changes were calculated using unstandardized coefficients from the regression analyses for each dependent variable on the respective categorical variables and contrasts. In all the analysis, type I error < 0.05 was chosen as the levels of statistical significance.

Results

Descriptives statistics (mean ± s.d.) for body composition and hormone levels of EX+HRT, HRT, EX and CONT groups at baseline are shown in Tables 12 and 13. No significant differences were found among the groups at baseline for any body composition variable. There were significant baseline differences between HRT and no HRT groups in E_1, E_2, GH and cortisol levels. The HRT groups had higher levels of E_1 and E_2 than the no HRT groups as expected. The EX+HRT group had higher levels of GH than the other three groups. The HRT group had significantly higher levels of cortisol than the EX and CONT groups, but not the EX+HRT group.

No significant difference in attendance was found at six months among the group of exercisers (EX+HRT vs EX and obese vs non-obese). From 6 to 12 months, there was a significantly lower attendance of the EX (71.2%) compared to the EX+HRT (83.7%) group. The overall attendance to the training program was lower for the last six months of the study.

Table 12. Baseline body composition for postmenopausal women in EX+HRT, HRT, EXERCISE and CONTROL groups.

	GROUPS							
	EX+HRT n=16		HRT n=22		EXERCISE n=20		CONTROL n=26	
Variables	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Weight (kg)	70.2	12.3	68.9	11.1	70.6	10.0	67.0	11.0
Total Body LST (kg)	39.8	4.8	38.8	4.3	39.7	4.6	37.7	4.3
Arm LST (kg)	3.9	0.8	3.7	0.6	4.0	0.7	3.7	0.6
Leg LST (kg)	13.0	1.7	12.5	1.6	12.9	1.5	12.2	1.5
Trunk LST (kg)	20.0	2.4	19.7	2.3	19.9	2.5	19.0	2.3
Leg LST/Fat	1.24	0.32	1.32	0.40	1.39	0.70	1.22	0.30
Total Body Fat (kg)	27.5	8.6	26.6	8.7	27.7	7.6	26.3	8.0
Arm Fat (kg)	2.5	1.0	2.4	0.9	2.5	0.9	2.3	0.9
Leg Fat (kg)	11.2	3.4	10.3	3.2	10.6	3.3	10.6	3.0
Trunk Fat (kg)	12.8	4.4	13.0	5.0	13.5	4.0	12.4	4.4
Trk/Leg Fat	1.14	0.21	1.27	0.34	1.35	0.47	1.18	0.28
Fat (%)	38.4	6.0	37.7	7.5	38.7	6.1	38.5	5.8

No significant differences among the groups.

Table 13. Baseline hormonal levels for postmenopausal women in EX+HRT, HRT, EXERCISE and CONTROL groups.

	GROUPS							
	EX+HRT n=16		HRT n=22		EXERCISE n=20		CONTROL n=26	
Variables	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Estrone (pg/mL)	128.6	85.7 [b,c]	151.9	69.4 [d,e]	21.0	12.7	15.6	8.0
Estradiol (pg/mL)	63.8	37.3 [b,c]	59.2	21.2 [d,e]	11.5	7.0	10.5	9.8
G H (ng/mL)	1.92	1.45 [a,b,c]	0.94	0.88	0.84	0.66	0.66	0.59
IGF-I (ng/mL)	139.1	35.5	120.5	53.8	151.7	52.6	139.1	35.5
A-4 (ug/dL)	1.55	0.65	1.81	0.66	1.72	0.57	1.73	0.68
Cortisol (ug/dL)	24.5	8.8	29.6	7.5 [d,e]	20.9	7.5	22.8	7.6

Abbreviations: SD= Standard Deviation, GH= Growth Hormone, IGF-I= Insulin-Like Growth Factor-I; A-4= Androstenedione.
[a] Significantly different EX+HRT vs HRT; [b] EX+HRT vs EX; [c] EX+HRT vs CONT; [d] HRT vs EX; [e] HRT vs CONT ($p < 0.05$).

The average increase in 1 RM at the end of the study was 98.5, 23.7 and 35.4% for leg press, seated row and military press, respectively (Figure 3). Increases in muscle strength at 12 months were all significant for the exercise groups with no difference between the EX+HRT and EX or obese and non-obese groups (Table 14).

Adjusted means and percent changes from baseline to 6 and 12 months in body composition are presented in Tables 15 and 16. Changes are adjusted for baseline, year of age, HRT use, exercise training and years postmenopause. Exercise training without HRT (EX) resulted in significant increases in LST of the total body, arms, legs, and trunk in the EX group at 6 and 12 months. In the EX+HRT group, the only significant increase in LST was in the arms at 6 months. Significant decreases in % fat were observed in the EX group at 6 (2.0%) and 12 months (1.8%). The leg LST/ leg Fat mass ratio was increased by exercise training in both EX and EX+HRT groups at 6 months. Changes in total and regional fat mass, fat distribution and body weight were not significant among groups ($p > 0.05$).

Adjusted means and percent changes from baseline to 6 and 12 months in hormone levels are shown in Table 17. Regardless of exercise status, significant decreases in E_2 levels at 6 and 12 months, and in GH levels at 12 months were found in both groups of women who were no HRT (EX and CONT). In the EX+HRT group, exercise training resulted in significant decreases in E_1 and E_2 at 6 months. There were no changes in GH, A-4 and cortisol levels in response to exercise training.

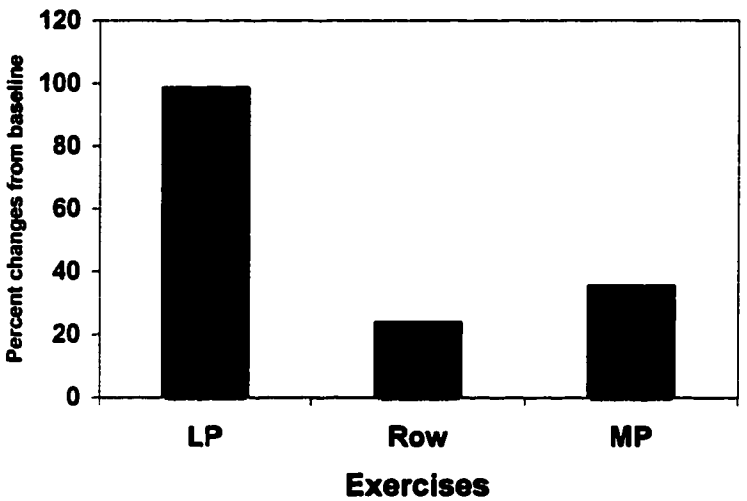

Figure 3. Percent changes from baseline in 1 RM in the exercising postmenopausal women.
LP= Leg Press, MP= Military Press.

Table 14. Baseline, 12 months, and changes (kg and %) means and standard deviation for 1 RM strength.

	Baseline		12 Months		12 Month Changes		Changes (%)	
	Mean, kg	SD	Mean, kg	SD	Mean, kg	SD	Mean	SD
EX+HRT								
Leg Press	94.7	17.1	185.8	26.3	91.1	9.2 *	96.2	54.2 *
Row	37.4	4.5	43.5	6.3	9.1	1.8 *	24.5	39.7 *
Military Press	8.5	2.4	12.2	2.2	3.7	0.2 *	43.9	10.0 *
EX								
Leg Press	97.4	35.3	195.4	49.1	97.9	13.9 *	100.5	39.4 *
Row	36.4	5.6	44.8	4.9	8.4	0.7 *	23.1	12.1 *
Military Press	9.2	2.9	11.8	2.4	2.6	0.5 *	28.5	16.9 *
Non-Ob EX								
Leg Press	97.3	26.6	185.1	33.2	87.8	6.6 *	90.2	24.7 *
Row	36.8	5.6	44.9	5.6	8.1	0.0 *	21.9	0.1 *
Military Press	8.7	2.6	11.7	2.1	3.0	0.5 *	34.3	19.1 *
Obese EX								
Leg Press	94.4	30.7	199.6	48.3	105.2	17.6 *	111.4	57.3 *
Row	36.8	4.1	46.9	5.5	10.1	1.4 *	27.2	33.1 *
Military Press	9.1	2.8	12.5	2.5	3.4	0.3 *	37.2	10.9 *

* Significantly differente from baseline ($p < 0.05$)

Table 15. Mean changes in Lean Soft Tissue at 6 and 12 months (kg and %) in EX+HRT, HRT, EXERCISE and CONTROL groups.

	GROUPS							
	EX+HRT		HRT		EXERCISE		CONTROL	
Variable	kg	%	kg	%	kg	%	kg	%
Total Body LST								
6 mo change	0.7	1.7	0.1	0.2	0.9	2.4 [b]	-0.4	-1.0
12 mo change	0.6	1.4	0.1	0.3	0.6	1.6 [b]	-0.6	-1.5
Arm LST								
6 mo change	0.2	6.3 [a]	0.1	1.7	0.2	4.8 [b]	0.0	1.0
12 mo change	0.2	5.1	0.1	1.6	0.2	4.4 [b]	0.0	0.1
Leg LST								
6 mo change	-0.1	-0.4	-0.1	-1.1	0.2	1.8 [b]	-0.3	-2.5
12 mo change	0.1	0.6	-0.2	-1.6	0.1	1.0 [b]	-0.2	-1.9
Trunk LST								
6 mo change	0.5	2.5	0.2	0.8	0.5	2.6 [b]	-0.2	-0.9
12 mo change	0.3	1.7	0.3	1.6	0.3	1.7 [b]	-0.3	-1.5
Leg LST/Fat								
6 mo change	0.1	4.4 [a]	-0.1	-3.4	0.1	9.0 [b]	0.0	0.4
12 mo change	0.1	9.1	0.0	-0.5	0.1	7.0	0.0	-0.2

Abbreviations: HRT= Hormone Replacement Therapy; EX+HRT= Exercise + HRT; LST= Lean Soft Tissue.

[a] EX+HRT vs HRT; b EX vs CONT ($p < 0.05$).

Variables were adjusted for HRT use, exercise, year of age, years postmenopause and baseline value.

Table 16. Mean changes in body fat at 6 and 12 months (kg and %) in EX+HRT, HRT, EX and CONTROL groups.

	GROUPS							
	EX+HRT		HRT		EXERCISE		CONTROL	
Variable	kg	%	kg	%	kg	%	kg	%
Total Body Fat								
6 mo change	-0.3	-1.2	0.5	1.7	-0.3	-1.2	0.4	1.5
12 mo change	-0.9	-3.2	0.2	0.8	-0.4	-1.5	1.2	4.6
Arm Fat								
6 mo change	0.0	0.2	0.1	2.7	0.1	2.4	0.1	5.4
12 mo change	-0.1	-5.2	0.1	5.3	0.1	4.6	0.2	7.7
Leg Fat								
6 mo change	-0.5	-4.1	0.1	1.1	-0.4	-3.7	0.0	-0.3
12 mo change	-0.6	-5.7	0.0	0.3	-0.2	-2.2	0.3	2.5
Trunk Fat								
6 mo change	0.1	0.8	0.2	1.9	0.0	-0.1	0.3	2.6
12 mo change	-0.2	-1.3	0.0	0.1	-0.3	-2.1	0.7	5.7
Trunk/Leg Fat								
6 mo change	0.05	4.3	0.01	0.9	0.05	3.9	0.00	3.7
12 mo change	0.04	3.2	0.02	1.4	0.02	1.6	0.04	3.5
Weight								
6 mo change	0.0	0.1	-0.2	-0.3	0.5	0.7	-1.1	-0.2
12 mo change	-0.2	-0.3	0.0	0.0	0.1	0.1	0.7	1.1
Fat (%)								
6 mo change	-0.6	-1.5	0.8	2.1	-0.8	-2.0 [a]	0.6	1.6
12 mo change	-1.3	-3.4	0.1	0.3	-0.7	-1.8 [a]	1.1	2.9

Abbreviations: HRT= Hormone Replacement Therapy; EX+HRT= Exercise + HRT; LST= Lean Soft Tissue.

[a] EX vs CONT ($p < 0.05$). Variables were adjusted for HRT use, exercise, year of age, years postmenopause and baseline value.

Table 17. Mean changes in hormone levels at 6 and 12 months in EX+HRT, HRT, EX and CONTROL groups.

	GROUPS							
	EX+HRT n=16		HRT n=22		EXERCISE n=20		CONTROL n=26	
Variables	mean	%	mean	%	mean	%	mean	%
Estrone (pg/mL)								
6 mo change	-17.9	-13.9 [a]	38.6	25.4	-2.6	-12.3	-7.0	-45.1
12 mo change	-19.0	-14.8	-11.0	-7.3	8.5	40.5	6.3	40.3
Estradiol (pg/mL)								
6 mo change	-0.5	-0.8 [a]	34.6	58.4	-9.5	-82.4 [b]	-12.8	-121.9 [b]
12 mo change	7.6	7.9	11.3	14.7	-7.7	-89.0 [b]	-11.2	-131.0 [b]
G H (ng/mL)								
6 mo change	0.19	10.1	0.20	22.2	-0.02	-2.5	-0.10	-15.3
12 mo change	0.51	26.4	0.11	11.4	-0.32	-38.5 [b]	-0.34	-52.0 [b]
A-4 (ng/mL)								
6 mo change	0.08	4.8	0.03	1.4	0.12	7.2	0.07	4.1
12 mo change	0.13	8.4	-0.05	-2.7	-0.01	-0.5	0.14	8.2
Cortisol (ug/dL)								
6 mo change	2.8	11.6	1.7	5.6	-0.1	-0.4	-0.5	-2.2
12 mo change	3.9	16.0	1.5	3.5	-0.2	-1.0	0.9	3.7

EX= exercise; HRT= Hormone Replacement Therapy; GH= Growth Hormone; A-4= Androstenedione.

[a] EX+HRT vs HRT; [b] HRT (HRT and EX+HRT) vs No HRT (CONT and EX) ($p < 0.05$).

Variables were adjusted for HRT use, exercise, year of age, years postmenopause and baseline value.

To analyze the potential influence of obesity on the hormone level and body composition changes to exercise training, further classification of the subjects in non-obese (n=50) and obese (n=34) subsamples resulted in the following subgroups: non-obese and no EX (n=28), non-obese and EX (n=22), obese and no EX (n=20), and obese and EX (n=14). Subjects were classified as obese with % fat > 40% as determined by DXA. Baseline characteristics (means ± s.d.) by obesity status are presented in Table 18. Compared to the non-obese group, the obese group had significant greater weight, BMI, LST (total body, arms, legs and trunk), fat mass (total body, arms, legs, and trunk), leg LST/Fat ratio, % fat, and serum levels of E_1 and E_2. Baseline values of GH were significantly higher for the non-obese EX group compared to the other groups (Table 19).

Adjusted means and percent changes from baseline to 6 and 12 months in LST and fat mass for non-obese and obese groups are presented in Tables 20 and 21, respectively. Regardless of exercise training, there were significant differences between the non-obese and obese groups in soft tissue composition changes of the legs. Both obese groups (no EX and EX) had significant decreases in leg LST at 6 months and in leg fat mass at 12 months. The leg LST/Fat ratio increased significantly in both obese groups at 12 months.

Exercise training had significantly different effects on LST and %fat in the non-obese and obese groups (Tables 20 and 21). Percent changes of total body LST increased significantly in the non-obese EX from baseline to 6 months (2.5%) but not to 12 months (1.4%). The obese EX group experienced significant increases from baseline in total body LST at 6 (1.6%) and 12 months (1.5%). Significant increases in arm LST were observed

in both the non-obese EX (5.4%) and obese EX (5.5%) groups at 6 months but increased significantly only in the obese EX (6.2%) at 12 months. The non-obese EX group increased significantly the LST of the legs at 6 (2.7%) and 12 months (1.5%). Trunk LST increased significantly only in the obese EX group at 6 months (3.1%). In the non-obese EX group, there was a significant increase in leg LST/Fat ratio at 6 (4.1%) and 12 months (7.8%). There was a trend toward a decrease in leg fat mass (8.0%) in the obese EX group at 12 months, and the exercise effect was almost significant (p=0.052). On the other hand, there was a significant decrease in changes of % fat in the non-obese EX group at 6 (-3.4%) but not at 12 months (-0.1%) compared to the non-obese no EX group. The obese EX group had the highest decrease in % fat (-4.6%) and the exercise effect was almost significant (p=0.067) at 12 months.

Adjusted means and percent changes from baseline to 6 and 12 months in hormone levels for non-obese and obese groups are presented in Table 22. Significantly lower levels of E_2 (-20.5%) were found at 6 months in the EX groups. Although percent changes in E_1 were –18.3% and –20.5% at 6 and 12 months, respectively, neither change was significant (p=0.07 and p=0.34, respectively).

Table 18. Baseline body composition for non-obese and obese postmenopausal women assigned to no exercise and exercise.

	GROUPS							
	NO EXERCISE				EXERCISE			
	NON-OBESE n=28		OBESE n=20		NON-OBESE n=22		OBESE n=14	
Variables	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total Body LST (kg)	37.3	4.3	39.6	4.0	38.8	5.4	40.8	2.8
Arm LST (kg)	3.6	0.6 [b]	3.9	0.6	3.8	0.8	4.2	0.5
Leg LST (kg)	11.9	1.7 [b]	13.0	1.3	12.7	1.8	13.4	1.0
Trunk LST (kg)	18.9	2.2	20.0	2.3	19.5	2.8	20.4	1.7
Leg LST/Fat	1.44	0.34 [a,b]	1.02	0.19 [c]	1.51	0.62 [d]	1.02	0.20
Total Body fat (kg)	20.8	4.2 [a,b]	34.3	5.5 [c]	22.3	4.4 [d]	35.6	4.9
Arm fat (kg)	1.8	0.6 [a,b]	3.1	0.7 [c]	1.9	0.6 [d]	3.4	0.7
Leg fat (kg)	8.6	1.9 [a,b]	13.1	2.5 [c]	9.0	2.2 [d]	13.7	2.8
Trunk fat (kg)	9.6	2.5 [a,b]	17.0	3.3 [c]	10.5	2.5 [d]	17.3	2.7
Trk/Leg fat	1.2	0.3 [a,b]	1.3	0.3 [c]	1.2	0.4 [d]	1.3	0.3
Fat (%)	33.7	4.1 [a,b]	44.4	3.5 [c]	34.6	3.8 [d]	44.5	3.1
Weight (kg)	61.5	7.7 [a,b]	77.0	8.2 [c]	64.1	8.7 [d]	79.8	6.6

[a] Significantly different non-obese no EX vs obese no EX; [b] non-obese no EX vs obese EX; [c] obese no EX vs non-obese EX; [d] non-obese EX vs obese EX ($p < 0.05$).

Table 19. Baseline hormonal levels for non-obese and obese postmenopausal women assigned to no exercise and exercise.

	GROUPS							
	NO EXERCISE				EXERCISE			
	NON-OBESE n=28		OBESE n=20		NON-OBESE n=22		OBESE n=14	
Variables	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Estrone (pg/mL)	70.2	79.6	84.8	89.0	60.5	69.5	88.6	92.1
Estradiol (pg/mL)	27.5	23.9	38.6	34.9	28.4	26.2	46.9	47.0
G H (ng/mL)	0.96	0.80 [a]	0.58	0.62 [b]	1.80	1.28 [c]	0.51	0.40
IGF-I (ng/mL)	143.5	47.2 [d]	111.1	35.9 [e]	146.2	51.6	119.6	39.5
A-4 (ug/dL)	1.68	0.69	1.86	0.65	1.74	0.57	1.52	0.65
Cortisol (ug/dL)	26.1	8.5	25.2	7.8	23.7	8.4	21.6	8.4

Abbreviations: SD= Standard Deviation, GH= Growth Hormone, A-4= Androstenedione.

[a] Non-obese no EX vs non-obese EX; [b] obese no EX vs non-obese EX; [c] non-obese EX vs obese EX ($p < 0.05$).

[d] Non-obese no EX vs obese no EX ($p=0.081$); [e] obese no EX vs non-obese EX ($p=0.070$).

Table 20. Mean changes in adjusted lean soft tissue at 6 and 12 months in non-obese and obese postmenopausal women assigned to no exercise and exercise.

	GROUPS							
	NO EXERCISE				EXERCISE			
	NON-OBESE n=28		OBESE n=20		NON-OBESE n=22		OBESE n=14	
Variables	kg	%	kg	%	kg	%	kg	%
Total Body LST								
6 mo change	0.0	0.1 [a]	-0.3	-0.7 [b]	1.0	2.5	0.6	1.6
12 mo change	0.0	0.0	-0.4	-1.0 [b]	0.5	1.4	0.6	1.5
Arm LST								
6 mo change	0.1	1.5 [a]	0.0	1.2 [b]	0.2	5.4	0.2	5.5
12 mo change	0.0	0.9	0.0	0.5 [b]	0.1	3.3	0.3	6.2
Leg LST								
6 mo change	-0.1	-1.1 [a]	-0.3	-2.2 [c]	0.3	2.7	-0.2	-1.5 [c]
12 mo change	-0.1	-1.1 [a]	-0.3	-2.1	0.2	1.5	0.0	-0.2
Trunk LST								
6 mo change	0.0	0.1	0.0	-0.2 [b]	0.4	2.0	0.6	3.1
12 mo change	0.1	0.6	-0.1	-0.4	0.3	1.5	0.4	1.9
Leg LST/Fat								
6 mo change	-0.07	-4.7 [a]	0.04	4.1	0.08	5.4	0.08	8.2
12 mo change	-0.09	-6.0 [a]	0.08	7.8 [c]	0.05	3.4	0.16	15.5 [c]

LST= lean soft tissue; mo=months.

[a] Non-obese no EX vs non-obese EX; [b] obese no EX vs obese EX; [c] Obese (no EX and EX) vs non-obese (no EX and EX) ($p < 0.05$). Variables were adjusted for obesity, exercise, year of age, years postmenopause and baseline value.

Table 21. Mean changes in adjusted fat mass and weight at 6 and 12 months in non-obese and obese postmenopausal women assigned to no exercise and exercise.

	GROUPS							
	NO EXERCISE				EXERCISE			
	NON-OBESE n=28		OBESE n=20		NON-OBESE n=22		OBESE n=14	
Variables	kg	%	kg	%	kg	%	kg	%
Total Body Fat								
6 mo change	0.3	1.5	0.4	1.3	-0.5	-2.4	0.0	0.0
12 mo change	1.7	7.9	0.0	0.0	0.5	2.1	-2.0	-5.6
Leg Fat								
6 mo change	0.3	3.6	-0.2	-1.8	-0.2	-1.7	-0.7	-5.0
12 mo change	0.7	8.4 [b]	-0.3	-2.5	0.1	1.4 [b]	-1.1	-8.0
Trunk Fat								
6 mo change	-0.1	-1.5	0.6	3.6	-0.5	-4.4	0.7	3.8
12 mo change	0.1	1.1	0.7	4.1	-0.3	-2.4	-0.3	-1.6
Trunk/Leg Fat								
6 mo change	0.01	0.8 [b]	0.05	3.5	0.02	1.4 [b]	0.09	6.8
12 mo change	0.01	0.6	0.06	4.3	0.01	1.1	0.04	3.3
Fat (%)								
6 mo change	0.6	1.9 [a]	0.5	1.2	-1.2	-3.4	0.1	0.2
12 mo change	1.7	4.9 [a]	-0.3	-0.6	0.0	-0.1	-2.1	-4.6
Weight								
6 mo change	-0.6	-0.8	0.2	0.3	0.3	0.6	0.1	0.1
12 mo change	0.4	0.7	0.6	0.7	0.3	0.4	-0.7	-0.9

[a] Non-obese no EX vs non-obese EX; [b] Obese (no EX and EX) vs non-obese (no EX and EX) ($p < 0.05$).
Variables were adjusted for obesity, exercise, year of age, years postmenopause and baseline value.

Table 22. Mean changes in adjusted hormone levels at 6 and 12 months in non-obese and obese postmenopausal women assigned to no exercise and exercise.

	GROUPS							
	NO EXERCISE				EXERCISE			
	NON-OBESE n=28		OBESE n=20		NON-OBESE n=22		OBESE n=14	
Variables	*units	%	*units	%	*units	%	*units	%
Estrone (pg/mL)								
6 mo change	1.8	2.6	31.6	37.3	-6.0	-9.9	-16.2	-18.3
12 mo change	-1.1	-1.6	4.4	5.2	-0.3	-0.5	-18.2	-20.5
Estradiol (pg/mL)								
6 mo change	8.3	30.1	13.4	34.8 [a]	-0.5	-1.8	-9.6	-20.5
12 mo change	0.1	0.4	-3.1	-8.0	-2.7	-9.5	-4.7	-10.0
G H (ng/dL)								
6 mo change	0.04	4.2	0.03	5.2	0.30	16.7	-0.09	-17.5
12 mo change	-0.03	-3.1	-0.12	-20.6	0.03	1.7	0.08	15.5
IGF-I (ng/dL)								
6 mo change	-2.83	-2.0	-8.75	-7.9	7.66	5.2	-5.44	-4.5
12 mo change	-2.91	-2.0	-0.31	-0.3	-0.35	-0.2	-9.43	-7.9
A-4 (ng/mL)								
6 mo change	0.03	1.8	0.11	5.9	0.17	9.8	-0.03	-2.0
12 mo change	-0.02	-1.2	0.19	10.2	0.18	10.4	-0.15	-9.9
Cortisol (ug/dL)								
6 mo change	1.97	7.5	-0.98	-3.9	1.59	6.7	1.34	6.2
12 mo change	0.28	1.1	2.12	8.4	2.80	11.8	0.40	1.9

GH= Growth Hormone; IGF-I= Insulin-Like Growth Factor-I; A-4= Androstenedione.

[a] Obese no EX vs Obese EX ($p < 0.05$).

Variables were adjusted for obesity, exercise, year of age, years postmenopause and baseline value.

Discussion

This study examined total and regional lean soft tissue and fat mass as well as hormonal levels in postmenopausal women on HRT and no HRT after 6 and 12 months of heavy resistance training program. The training program led to gains in LST of total body (2.3%), arms (5.0%) and legs (0.8%) compared to non-exercise women (HRT and no HRT). Gains in LST over the first 6 months were maintained but not significantly improved in the exercisers. A significant lower attendance to the last six months (76.9%) compared to the first six months (84.1%) of the training program may account for some of the difference.

We observed that exercisers not on HRT increased LST mass in arms, legs, and trunk, with the greatest increase occurring in the arms (4.8%). Women on EX+HRT increased arms but not legs and trunk LST. These results confirm previous studies in men and women that have shown that muscle of the arms respond more than muscle of the legs to the overload imposed by resistance exercise training (Welle et al., 1996; Cureton et al, 1988; Chilibeck et al., 1996). Similarly, using DXA, Lohman et al. (1995) and Chilibeck et al. (1998) reported that resistance exercise training resulted in greater gains in the arms than the legs LST in premenopausal women. In addition, Taaffe et al. (1995) reported a similar increase in arms LST mass (4.8%) with no change in whole body and legs LST in healthy non-obese women aged 65 to 79 years after 15-week heavy resistance training program (80% 1 RM). It seems that in untrained people with average levels of physical activity, LST of the arms appears to respond to a greater extent than

LST of the legs to heavy resistance exercise training suggesting that leg muscles are more accustomed to physical activity than arms.

We found the responses to resistance exercise training also varied with initial body composition. For example, there was an increase in leg LST of 1.0 to 1.8% in non-obese but not in obese women following 6 months of exercise training. No direct comparison between non-obese and obese subjects has been made in previous studies. Because some of the differences in the magnitude of body composition changes with exercise training can be explained on the basis of differences in initial values, our subjects were classified as non-obese and obese and subsequently in no exercise and exercise groups. Although the obese exercise subjects increased LST of the arms similarly to the non-obese exercise individuals, no significant changes associated with exercise training were observed in total body, legs and trunk.

Several studies support our findings in obese versus non-obese individuals. Subjects with low LST mass are the most likely to increase LST with exercise training. A factor that could explain this difference is the mechanical overload to which obese subjects are chronically accustomed. In addition, obese subjects had higher LST than non-obese at baseline. Forbes (1987) has shown that approximately 25% of the excess weight is lean body mass in the obese.

We observed a 2.2 % (obese no exercise) and 1.5% (obese exercise) decrease in LST of the legs that is similar to that found by Ross et al. (1995) for obese premenopausal women after 16-wk resistance exercise training plus diet. Although in our subjects there was no change in body weight from baseline to after 12 months of

resistance exercise training, it cannot be ruled out for certain that the loss in LST and fat mass in the obese group was associated with a reduction in caloric intake. Seven women in the obese no exercise group had a decrease in leg LST between 0.6 and 1.2 kg. On the other hand, just two of the obese exercisers had decreases in leg LST higher than 0.5 kg. The negative result in leg LST is consistent with the finding that caloric restriction in obese young women results in a greater loss of FFM and fat mass (Sweeney et al., 1993). However, a moderate food restriction in addition to a training program that combined aerobic and / or resistance exercises maintained the lean tissue and promoted the loss of fat mass in obese pre- and postmenopausal women and middle-age man (Sweeney et al., 1993; Svedensen et al., 1993; Ross et al., 1996). The finding in the changes in legs LST between the obese and non-obese is attributed to a reduction in the obese control group and to an increase in the non-obese exercise group.

In contrast to the significant overall increases in LST, significant decreases in total body, arms, legs and trunk fat mass were not found with exercise. Because aerobic exercise requires a minimal caloric expenditure of 1000-1200 kcalories to be effective in inducing weight loss (American College of Sports Medicine, 1995), we expected only small changes in total body fat mass or body weight with our training program. The EX+HRT group showed non-significant ($p < 0.09$) decreases in leg fat mass at 6 (2.2%) and 12 months (5.7%). Decreases in fat mass of the legs in the non-obese exercise group (-1.7%, $p=0.09$) nor in the obese exercise group (-8.0%, $p=0.052$) in response to exercise at 6 and 12 months, respectively, were also almost significant. With resistance training, Treuth et al. (1995a) found a significant 5.7% decrease in thigh fat area in older women.

Treuth et al. (1994) also found that energy expenditure during resistance exercise training is very low. However, obese women rely more on aerobic metabolism than lean women during intense exercise (Ardévol et al., 1998), and exercise training increases fat oxidation in women (Treuth et al., 1995b) and thus some fat loss may be elicited in obese women with resistance training. In the present study however women were not encouraged to loose weight and were instructed to maintain the same nutritional intake through the study period.

Our data indicate that the group of obese women had significantly greater LST/Fat mass ratios at 12 months. Decreases in LST occurred, together with greater reductions in fat mass of the legs in this group regardless of exercise training. Although the obese exercise group had greater decreases in fat mass and lesser decreases in LST of the legs, there were no significant differences with the obese no exercise in these variables. A higher decrease in fat mass than in LST mass in the legs of the obese exercising subjects suggest that LST of the legs was to some degree preserved with exercise training. Svedensen et al. (1993) reported that the addition of combined aerobic and resistance exercise to a reduced caloric intake resulted in the loss of fat mass (-9.6 $\pm$ 2.7 kg) with no significant changes in LST (0.0 $\pm$ 1.7 kg). Although they concluded that their resistance exercise training prevented the loss of LST in overweight postmenopausal women, 24 of the 48 subjects in the resistance exercise training plus diet group had a decrease in LST. Our results are consistent with results from recent studies where resistance exercise training reduced the loss of LST in obese subjects on restrictive diet (Ross et al., 1996; Geliebter et al., 1997). Furthermore, it is possible to observe

hypertrophy of the muscle fibers concurrently with fat mass and FFM loss in obese women when resistance exercise training and diet are combined (Donnelly et al., 1993).

Exercise training-changes in body composition might be associated with adaptations in some hormones (Thompson et al., 1998). This was our hypothesis and the reason for following changes in E_1, E_2, A-4, GH, IGF-I, and cortisol levels after 6 and 12 months of resistance exercise training. However, no change in the hormonal milieu related to exercise training was found except for both estrogens. Compared to the HRT group, the EX+HRT group showed significant decreases in E_1 (-13.9%) and in E_2 levels (-1.0%) at 6 months. The decrease in E_2 levels (-20.5 %) was also observed in the obese exercisers and may be explained by reduction in fat mass of the legs ($p < 0.07$), an important site for the aromatization of estrogens. E_2 has been positively associated with obesity (Vermeulen & Verdonck, 1978; Tazuke et al., 1992), and reduction of both E_1 and E_2 levels has been observed after weight reduction (Parker, 1989). This finding was not observed either in no HRT women or in non-obese women on exercise who had lower levels and lower variation in estrogen levels and no significant changes in either total body or regional fat mass.

The hormonal mechanisms to explain the increases in LST following heavy resistance exercise training programs are unclear. In our study, the lack of change in serum GH levels after resistance exercise training is consistent with other findings in young women (Staron et al., 1994) and older men and women (Pyka et al., 1994b). We found that non-obese women on HRT had significantly higher levels of GH compared with non-obese no HRT and obese HRT women. We also found that regardless of HRT

use, non-obese exercise women had significant gains in LST of arms and legs. Besides the attenuation in GH secretion observed with aging (Pyka et al., 1994b; Corpas et al., 1993a), obesity is another factor that may influence a blunted GH response to exercise (Williams et al., 1984; Dawson-Hudges et al., 1986). Decreased GH and IGF-I levels are important hormonal abnormalities that characterize obese people (Williams et al., 1984; Rudman et al., 1981; Marin et al., 1993). These findings may explain the lack of significant gain in leg LST in exercise obese women. Recent studies have shown that HRT or GH administration combined with exercise have not resulted in a greater increase in LST in older non-obese women and men (Brown et al., 1997; Yarasheski et al., 1995). However, it has been demonstrated that GH administration to obese postmenopausal women (5 of 7 on HRT) participating in resistance exercise training resulted in greater gains in LST than exercise with no GH therapy (Thompson et al., 1998). These results suggest the possibility that hormone therapy (either HRT or GH) is less effective in lean older people who have the capacity to secrete anabolic hormones in response to an acute exercise stimulus. In contrast, obese postmenopausal women on HRT or GH therapy have a blunted GH response to acute heavy resistance exercise that may explain in part the smaller increase in LST to resistance exercise training in these individuals. It is clear that HRT does not elicit gains in LST or losses in fat mass more than resistance exercise training alone.

The lack of change in A-4 levels is consistent with the findings of others (Hetrick & Wilmore, 1979; Staron et al., 1994; Hakkinen and Pakarinen, 1994), who reported no significant change in androgen levels in young and older women after resistance training.

Westerlind et al. (1987) observed significant decreases in A-4 levels for both the control and resistance exercise group after 10-wk training program. Changes in both groups suggest that factors other than training influenced their result. A-4 is important not for its androgenic potency but for its peripheral conversion into E_1, the most abundant estrogen in postmenopausal women. Decrease in fat mass of the legs was accompanied by a significant reduction in E_1 levels in the EX+HRT group with no significant increase in A-4 levels (4.8%). Although adipose tissue from the thighs converts more A-4 to E_1 than abdominal fat (Bulun & Simpson, 1994), no significant change in A-4 levels was observed in any group. Because the exercise no HRT group demonstrated a greater decrease in E_1 levels than the EX+HRT group ($p > 0.05$), these results suggests that E_1 was not mainly originated from A-4 but from HRT. Conjugated equine estrogens that is a mixture containing mainly E_1 (50%) was the most used form of HRT in postmenopausal women in this study.

Although women on HRT had significantly higher baseline levels of cortisol, no significant change was observed over the training period. Similar findings have been reported in young, middle aged and older women after 6 to 12 weeks of resistance exercise training (Staron et al., 1994; Hakkinen & Pakarinen, 1994). In addition, Hakkinen et al. (1987 and 1988) found no change in serum cortisol to strength training in male elite weight lifters after one and two-year follow-up. Decreased cortisol levels may occur in adaptation to resistance exercise training that favors muscle hypertrophy in man (Hakkinen et al., 1987; Alen et al., 1988; Staron et al. 1994). In contrast, when high exercise intensity is maintained for 6 months, a plateau phase in strength gains is

observed even in young men (Hakkinen et al., 1985). These authors attributed this phenomenon to overtraining characterized in part by increased levels of cortisol. In our study, the exercise training program did not evoke the attenuated cortisol response also observed in young women after 8-12 weeks of heavy resistance exercise training (Kraemer et al., 1997 and 1998; Staron et al., 1994). The resistance exercise training in our study was not intense enough to result in overtraining and consequently increase cortisol levels. Therefore, is not likely that overtraining may be an explanation for the plateau in LST gains observed in the exercise groups during the last six months of the study.

In conclusion, there were no significant differences between women on HRT and women not on HRT in gains in LST in postmenopausal women, as HRT plus exercise had similar increases in LST in the arms, whereas exercise alone also improved LST in total body, legs and trunk. The HRT group did not show a loss in fat mass, whereas exercise training alone (but not in combination with HRT) was effective in % fat reduction. In addition, considering obesity, the results indicate that exercise training is effective in increasing LST of the arms and in preventing the decrease in LST of the legs observed in obese postmenopausal women. Changes in resting levels of the anabolic and catabolic hormones assessed in this study are not modified by exercise training and their chronic elevation may not be necessary for the positive adaptations in body composition observed in postmenopausal women not on HRT.

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