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SEX HORMONE LEVELS AND REGIONAL ADIPOSITY IN FEMALE SMOKERS

by

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In Partial Fulfillment of the Requirements

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ABSTRACT

The distribution of the adipose tissue mass is a better predictor of health risk than overall adiposity. Sex steroids are involved in determining and regulating the distribution of adipose tissue. Androgenic profiles relate to android (abdominal) adiposity, estrogenic profiles to gynoid (gluteal-femoral) adiposity. Women of reproductive viability tend to be gynoid, while men tend to be android; android adiposity represents much greater health risk than gynoid adiposity. Limited evidence suggests increased androgenic relative to estrogenic activity in female cigarette smokers, and smokers of either sex are characterized by greater degrees of android adiposity than nonsmokers. There is, however, no known mechanism for an effect of smoking on the distribution of body fat. It was hypothesized that cigarette smoking might interact with androgen/estrogen levels and adipose tissue distribution in reproductively capable women, thus providing some rationale for an association with android adiposity. Relationships between indices of regional adiposity -- waist-to-hip girth ratio (WHR), waist-to-thigh girth ratio (WTR) and waist girth-to-hip breadth ratio (WHbR) -- and serum concentrations of testosterone, estradiol and sex hormone-binding globulin (SHBG) were studied in 27 premenopausal smokers and 29 premenopausal nonsmokers. All variables were adjusted for overall fatness. Smokers were characterized by significantly greater degrees of android adiposity (elevated WHR, WTR and WHbR) and greater serum concentrations of SHBG than nonsmokers. There were no significant differences between groups for testosterone or estradiol levels, although testosterone was relatively greater in the smokers, and estradiol relatively lower. There were significant interactions between smoking, testosterone levels and both WHR and WTR; the relative effect of testosterone on android adiposity was greater in smokers. The data confirm (a) the association of smoking with abdominally localized body fat and (b) an earlier observation of elevated serum SHBG in premenopausal smokers (which has not been adequately studied), contrary to directional differences in testosterone and estradiol. The data demonstrate, for the first time, an interactive effect of cigarette smoking with serum testosterone levels and regional adiposity. Such a finding is of biological significance in that smoking appears to predispose premenopausal women toward a more masculine distribution of body fat, one which is clearly linked to health risk, via effects on sex steroids.

DEDICATION

This work is dedicated to Donna for her love, patience and understanding.

Read not to contradict and confute, nor to believe and take for granted, nor to find talk and discourse, but to weigh and consider.

- Francis Bacon

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LIST OF ABBREVIATIONS

A ..... androstenedione
ACTH ..... adrenocorticotropin
ANCOVA ..... analysis of covariance
ANOVA ..... analysis of variance
BMI ..... body mass index
cAMP ..... cyclic adenosine monophosphate
CBG ..... corticosteroid-binding globulin
CT ..... computed tomography
DHEA ..... dehydroepiandrosterone
DHEA-S ..... dehydroepiandrosterone sulfate
DHT ..... dihydrotestosterone
E_1 ..... estrone
E_1S ..... estrone sulfate
E_2 ..... estradiol
E_2S ..... estradiol sulfate
E_3 ..... estriol
FAI ..... free androgen index
FEI ..... free estrogen index
FFA ..... free fatty acids
FIA ..... fluoroimmunoassay
FSH ..... follicle-stimulating hormone
GH ..... growth hormone
GnRH ..... gonadotropin-releasing hormone
IGF-1 ..... insulin-like growth factor-type 1
LH ..... luteinizing hormone
LHRH ..... luteinizing hormone-releasing hormone
ß-LPH ..... (beta) lipotropin
LPL ..... (adipose tissue) lipoprotein lipase
NADPH ..... nicotinamide adenine dinucleotide phosphate
NIDDM ..... non-insulin-dependent diabetes mellitus
NMRI ..... nuclear magnetic resonance imaging
O_2 ..... molecular (di)oxygen
PCOS ..... polycystic ovarian syndrome
RIA ..... radioimmunoassay

SE(M).....standard error (of measurement)
SHBG .....sex hormone-binding globulin
$\propto SSF_c$.....proportional sum of central skinfolds
$\propto SSF_o$.....proportional sum of overall skinfolds
T.....testosterone
UHR.....umbilical-to-hip girth ratio
VLDL..... very low density lipoprotein
WHR..... waist-to-hip girth ratio
WHbR.....waist girth-to-hip breadth ratio
WTR..... waist-to thigh girth ratio

CHAPTER 1: INTRODUCTION

Obesity, Adipose Tissue Distribution, and Associated Health Risks

Health Implications of Obesity

Obesity is a serious health problem in contemporary society. Defined as an excess of body fat, obesity differs from overweight in that overweight is defined as an excess of body weight over some arbitrary standard defined in relation to height. Because assessment of fat is impractical in population studies, most estimates of the prevalence of obesity are actually estimates of the prevalence of overweight. The Body Mass Index (BMI, or Quetelet Index) is most often used as an estimate of obesity; it is obtained by dividing body mass in kilograms by the square of stature in meters (kg/m^2). Although there are theoretical reasons for using the terms "body mass" and "stature", the popular analogues of "weight" and "height" will be considered to be interchangeable with these terms from hereon (Ross W.D. et al., 1987). There is some controversy regarding whether or not a sample-specific exponent is a better dissociator of weight than height squared, and various other forms of weight and height indices are available (Abdel-Malek et al., 1985). Regardless, it has been contended that it makes little difference which of these indices is used in the clinical appraisal of obese adults because relative adiposity, however defined, ends up being virtually identical (Collvier et al., 1983). Though there is considerable support for the BMI as an index of obesity in population studies, it is not valid for individual assessment (Ross W.D. et al., 1987). It is a measure of ponderosity, as much a measure of lean body mass as fatness and is also dependent upon body proportions (Garn et al., 1986b).

The BMI has been used to estimate the prevalence of obesity in Canada and the United States (Bray, 1989). The normal range for the BMI is 20 to 25 kg/m^2. Overweight is considered to compose the range of BMI from 25 to 30 kg/m^2 for both males and females. Within this category males range from 31% to 40% and females from 24% to 28% of the population. Very overweight (or obese) individuals, identified by BMI greater than 30 kg/m^2, range from 9% to 12% of the population.

Overweight is associated with health complications, and this association has been reported in both longitudinal and cross-sectional studies. Large population studies (Build and Blood Pressure Study 1959, 1960; Build Study 1979, 1980; Lew & Garfinkel, 1979; Waaler, 1983) have yielded consistent results which have been confirmed by numerous smaller studies. This overall relationship between BMI, mortality and disease has been well-described by the above-mentioned review (Bray, 1989). Figure 1-1 illustrates this

relationship, which is best characterized as a J- or U-shaped curve. For both sexes, minimum mortality is characterized by a BMI of 22 to 25 kg/m^2. Increased mortality is associated with BMIs above or below this range (Manson et al., 1987). As denoted by the lowest mortality range of BMI between 20 and 25 kg/m^2, it is apparent that relatively small fluctuations in weight have little effect on life expectancy. Mortality increases at a BMI of 30 kg/m^2, and at a BMI of 40 kg/m^2 mortality is greatest. Conversely, mortality also increases at a BMI of less than 20 kg/m^2. These two end-points of the U-shaped distribution between relative weight and mortality represent different causes of death. Digestive diseases, respiratory diseases and cancer compose the low BMI deaths. High BMI causes of death are cardiovascular diseases, diabetes mellitus, gallbladder disease and some cancers. It is notable that minimum mortality has been registered at a BMI close to 25 kg/m^2 in both men and women (Waaler, 1983).

The high mortality rate associated with lower-than-average BMI is a curious phenomenon. Perhaps people characterized by low BMI are already ill and therefore have an elevated mortality ratio, but high mortality rates persist even when illness is taken into consideration, as in the above-noted studies. Another observation is that pulmonary disease, dominating the low BMI mortality has a much lower mortality ratio than the diseases characterized by high BMI, in which the major cause of death is cardiovascular disease. The relationship between BMI and cardiovascular disease has been examined in isolation from other causes of mortality (Figure 1-2), and results have shown a curve

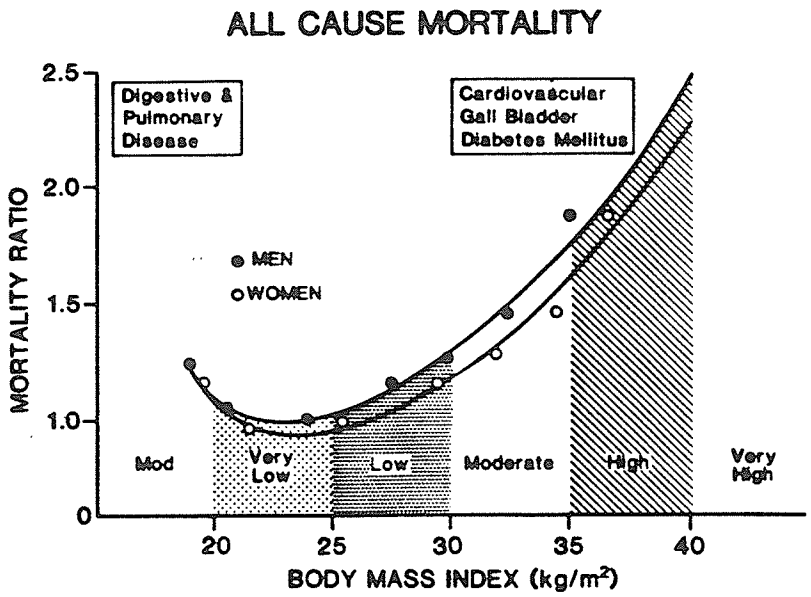

FIGURE 1-1. All Cause Mortality and Body Mass Index. Copyright 1987 George A. Bray used with permission.

which is also J- or U-shaped, with an increase in mortality at both high and low BMIs (Waaler, 1988). This increase of cardiovascular disease at low BMIs is interesting, and will be returned to shortly, even though the characteristics of those who die from cardiovascular diseases at a low BMI versus those at a high BMI are not known.

Discrepancies such as these suggest that guidelines of desirable weight based on BMI are not very meaningful. They might, however, be of use in defining obesity as a risk factor, but that is all. Problems arise when examining the relationship of BMI with specific diseases. For example, there is universal agreement between the positive association of BMI and non-insulin-dependent diabetes mellitus (NIDDM) (Björntorp, 1988b) but with other diseases, specifically cardiovascular disease, the relationship with BMI is much more complex (as noted above). Associations of *low* BMI with a high risk of cardiovascular disease (Waaler, 1988; Larsson, 1988) suggest that: (a) underweight is associated with increased mortality; and (b) a slight degree of overweight may actually be "healthy". If this is so, then it follows that the established relationship between obesity and health risk is unclear. Perhaps the only point which is clear is that at best, obesity is only a crude measure of overall health risk. Means other than estimates of obesity are needed to better assess health risk.

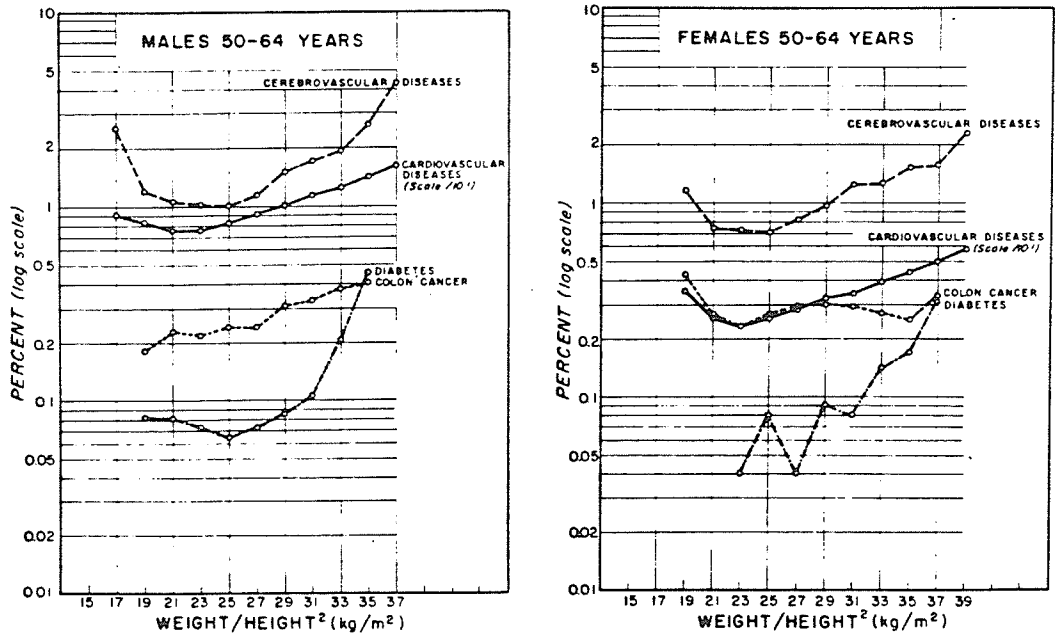

FIGURE 1-2. Relationship of Specific Disease States to Mortality by BMI. From Waaler, H. T. (1988). Hazard of obesity--the Norwegian experience. Acta Medica Scandinavica, 723(Suppl), 17-21. Reproduced with permission.

Health Implications of Regional Adiposity

Rather than look at overweight from a quantitative point of view, it might be better to qualify the degree of overweight. Thus, the distribution of adipose tissue becomes a factor of obvious significance. The concept was first introduced in the 1950s by Vague, who described differences in adipose tissue distribution in relation to metabolic and cardiovascular disorders. He described "gynoid" obesity as being characterized by lower body predominance, "menaced only by direct mechanical complications of excessive adiposity: locomotor difficulties, abdominal pressure, limitation of respiratory motion, slowing of the venous and lymphatic circulation, cellulitis, lowering of energy, and reduction of the elasticity of the fat-infiltrated myocardium -- complications which are all proportional to the degree of excess fat" (Vague, 1956, p. 31). "Android" obesity was described as having upper body predominance, and leading to metabolic disturbances such as diabetes, gout, uric calculous disease and premature atherosclerosis.

Largely ignored for many years, the observations of Vague (1956) regarding the importance of the regional distribution of adipose tissue have received renewed interest over the last decade. During this time, the ratio of waist circumference to hip circumference (WHR) became validated as an independent risk factor for premature death, cardiovascular disease, stroke, NIDDM and some female carcinomas (Björntorp, 1988b). The WHR assesses the degree of android adiposity relative to gynoid adiposity independent of degree of obesity; assessed health risk is also independent of degree of obesity (Björntorp, 1988b). In view of the previously-noted discrepancies of the BMI with certain diseases, this is an important point.

The two types of adipose tissue distributions proposed by Vague have been accepted. Android adiposity is also known as abdominal, central, centripetal, truncal, upper-body, or male-type; gynoid adiposity is also known as gluteal-femoral, peripheral, centrifugal, lower-body, or female-type. There have been five prospective studies reported which have examined the relationship of fat distribution with mortality and disease in both men and women (Lapidus et al., 1984; Larsson et al., 1984; Ducimetière et al., 1986; Stokes et al., 1985; Donahue et al., 1987). The WHR was the primary method utilized to assess body fat distribution, although other methods such as skinfolds and skinfold ratios were also used. As recently reviewed (Bray & Gray, 1988), all studies found abdominal (android) fat predominance to be a potent risk factor for cardiovascular disease, hypertension, stroke and diabetes. Android fat distribution was found to be a greater risk factor than BMI; this was how the WHR was validated, as a risk factor independent of BMI. The relationship of WHR with mortality is illustrated in Figure 1-3 (Lapidus et al., 1984).

Data are divided into fifths (quintiles) of the population studied. The quintile with the lowest WHR shows a much greater chance of remaining free of myocardial infarction and of long-term survival compared to the quintile with the highest WHR (Lapidus et al., 1984). Notably, this effect was independent of total fat, indicating that the regional pattern of the adipose tissue distribution is of importance in the overall health risk profile of both normal and obese individuals.

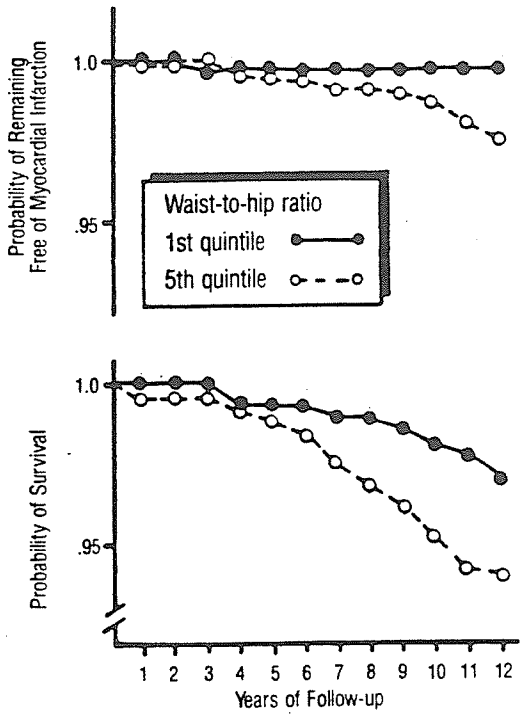

FIGURE 1-3. Cumulative Mortality in Relation to WHR. From Lapidus, L., Bergtsson, C., Larsson, B., Pennert, K., Rybo, E. & Sjostrom, L. (1984). Distribution of adipose tissue and risk of cardiovascular disease and death: a 12 year follow up of participants in the population study of women in Gothenburg, Sweden. British Medical Journal, 289, 1257-1261. Reproduced with permission.

Supporting the above data are many cross-sectional studies. In both sexes, abdominal fat predominance is again associated with glucose intolerance, hyperinsulinemia and hyperlipidemia (Krotkiewski et al., 1977; Smith U. et al., 1979; Krotkiewski et al., 1983; Kalkhoff et al., 1983; Evans et al., 1984; Craig et al., 1968; Feldman et al., 1969; Després et al., 1988b) and hypertension (Blair et al., 1984; Hartz et al., 1984; Troisi et al., 1990). Centrally localized adipose tissue has even been implicated in cases of endometrial and ovarian carcinoma, and incidence data suggest that analysis of anthropometric variables indicating central adipose tissue distribution may be of predictive value for these malignancies (Lapidus et al., 1988). Conversely, gynoid adipose tissue distribution is not directly associated with those health risks imposed by central distribution of adipose tissue (Blair et al., 1984; Lapidus et al., 1988). Figure 1-4 shows relative risk for percentiles for the WHR depicted for men and women by age groups. Relative risk increases with increasing WHR, reflecting greater android adiposity.

Pathogenesis of Complications Associated with Regional Adiposity

This is not intended to be a comprehensive review of the possible mechanisms by which abdominally-located adipose tissue is linked to disease states. Suffice it to say that the area is extremely complicated and much remains to be uncovered.

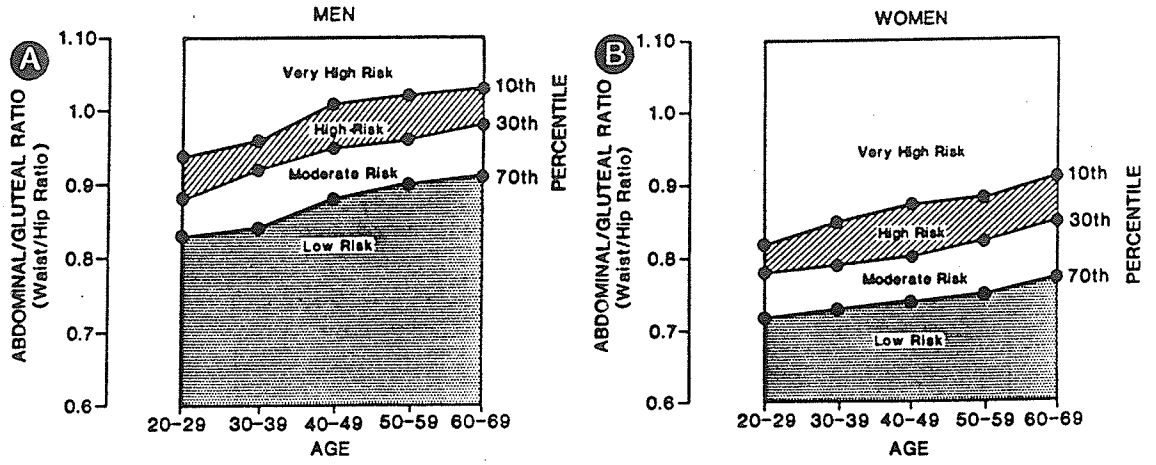

FIGURE 1-4. Health Risk, by Percentile, According to Age and WHR. Copyright 1987 George A. Bray used with permission.

It is possible that an increased mass of abdominal tissue is causally associated with disease. Abdominal adipose tissue, particularly intra-abdominal, has high lipolytic activity (Smith U. et al., 1979), and enlarged abdominal adipocytes further exaggerate production of excess free fatty acids (FFA) into both the general and portal circulation (Kissebah et al., 1985). This may relate to decreased α to ß adrenergic activity (Kissebah et al., 1982). Negative effects of excess FFA in circulation are: (a) decreased glucose transport and insulin effectiveness (Peiris et al., 1987b); (b) increased plasma triglycerides and cholesterol through effects of FFA on the liver, with increased secretion of very low density lipoproteins (VLDL) (Björntorp, 1988b); and (c) increased plasma insulin (Kral et al., 1977). There are strong associations of excess plasma FFA with hypertension and hyperinsulinemia (Peiris et al., 1989; Thiébaud et al., 1982).

Alternately, it is possible that abdominal adiposity is one of several secondary consequences of increased secretion of androgens and cortisol from the adrenal cortex, and low levels of estrogens and sex hormone-binding globulin (SHBG) (Holm & Krotkiewski, 1988). It has been proposed that this might cause acute and/or chronic functional and morphological changes in muscle, such as insulin resistance, these being prerequisites for the precipitation of NIDDM and hypertension (Björntorp, 1988b). White, fast twitch fibres are less insulin sensitive than red, slow twitch fibres as measured in both *in vitro* (Wallberg-Henriksson, 1987) and *in vivo* (Hom & Gooder, 1984; Björntorp, 1988b) investigations, and both men and women with high WHR have muscle fibre compositions characterized by a high proportion of white, fast twitch, Type IIb fibres (Krotkiewski & Björntorp, 1986; Björntorp, 1985) in combination with low capillary density (Holm &

Krotkiewski, 1988). Furthermore, it is possible that androgens and cortisol might cause accumulation of fat in the abdominal region (Björntorp, 1988b).

Finally, a third possibility concerning neuro-endocrine dysregulation due to hypothalamic arousal has been proposed (Björntorp, 1988b). This model postulates that several endocrine axes are disturbed by the primary pathogenic factor of neuro-endocrine dysregulation at the hypothalamic level; standardized stress has been found to produce these disturbances in mice, primates and humans (Björntorp, 1988b). Thus, environmental stress might induce engagement of the sympatho-adrenal axis (causing hypertension and elevated FFA), the pituitary-adrenal axis (causing increased secretion of adrenal steroids), and the pituitary-ovarian axis (causing altered sex steroid levels, anovulation and abdominal adipose tissue distribution), therefore allowing development of most risk factors for stroke, cardiovascular disease, NIDDM and female cancer (Björntorp, 1988b).

It is not likely that any of these three possible mechanisms would work in isolation from the others. Any combination may be involved and, while there is considerable support for each mechanism, none alone can explain all of the conditions seen in individuals with android body fat.

Assessment of Regional Adiposity

Methods

While the WHR is the most widely used method of indexing android adipose tissue distribution, several other indices have also been used. These have included both circumferential and subcutaneous skinfold thickness measurements (Blair et al., 1984; Shimokata et al., 1989; Ashwell et al., 1982; Mueller et al., 1987), but circumferences are thought to be more reliable than skinfolds (Mueller & Malina, 1987). While central skinfold measurements are more closely associated than peripheral skinfold measurements with hyperlipidemia, hypertension and diabetes (Butler et al., 1982; Shear et al., 1987; Stallones et al., 1982; Peiris et al., 1988), visceral fat is not accurately reflected by skinfold measurements, and it is evident that both internal and subcutaneous abdominal fat are hazardous to health (Lapidus et al., 1984; Larsson et al., 1984). However, although circumferences are thought to be more reliable than skinfolds to assess fat distribution in adults, their validity as measures of such in pre-adults is not known (Mueller & Malina, 1987). It has been suggested that the waist-to-thigh circumference ratio (WTR) is a better method of assessing central fat in pre-adults than the WHR (Mueller et al., 1990).

In adults, the WHR is considered by many to be the best and simplest method (Krotkiewski et al., 1983; Kissebah et al., 1985; Ashwell et al., 1978; Del Ponte et al., 1989; Evans et al., 1983; Haffner et al., 1986b; Peiris et al., 1988) of indexing android adiposity and, consequently, predicting the health risk imposed by centrally located body fat. It is highly correlated with internal/visceral fat mass as determined by computed tomography (CT) (Borkan et al., 1982; Ferland et al., 1989), and computed tomography is a valid method for quantifying the size of subcutaneous and visceral fat depots (Enzi et al., 1986; Borkan et al., 1982; Grauer et al., 1984). The abdominal and subscapular skinfolds have also been shown to correlate significantly with intra-abdominal fat as determined by CT, although to a much lesser extent than WHR (Ferland et al., 1989). Recently, photon absorptiometry has been used to directly assess abdominal fat percentage, and the WHR also correlates significantly with this new *direct* technique (Schlemmer et al., 1990).

Many studies have shown that the WHR is significantly associated with diabetes, hypertension, glucose intolerance, cardiovascular disease and gallbladder diseases (Hartz et al., 1984; Larsson et al., 1984; Lapidus et al., 1984; Peiris et al., 1988; Ohlson et al., 1985) independent of degree of obesity. As a predictive index, it appears that health risks increase at a WHR of 0.80-0.85 for women and 0.95-1.00 for men (Björntorp, 1985; Peiris et al., 1989). Correlations of WHR to metabolic aberrations range from 0.45 to 0.60 ($p<0.05$) (Peiris et al., 1988). Unfortunately, the method for deriving this index has not been standardized, but there appears to be current consensus for using either minimum waist to maximum hip circumference or, in lieu of well-defined regions, halfway between the manubrium sterni and the umbilicus (in the case of a markedly dislocated umbilicus) and at the widest part of the gluteal regions (Björntorp, 1985; Peiris et al., 1989; Peiris et al., 1988).

Considerations

Defining fat distribution poses two methodological problems (Garn et al., 1988). The first pertains to fat distribution independent of total amount of fat, since relative thickness of outer fat varies with the total amount of fat. This is reflected by skinfolds and skinfold ratios, which are thus fatness-dependent. The second problem pertains to any ratio of circumferences -- such as WHR -- since either or both circumference variables may change as the total amount of fat changes with fluctuations in weight. The words "outer (subcutaneous) fat" may well be substituted for "total fat" because it has been demonstrated that most of the fat in humans is indeed outer fat (Martin et al., 1985). A major problem with both skinfolds and circumference measurements, then, is their stability over time. It is necessary to know how fat distribution is affected by various factors, and whether these

factors effect differential redistribution of fat. Some of these considerations are outlined below.

Weight Change

In general, women, but not men, have been found to be remarkably consistent in skinfold thicknesses over time and, adjusting for total fatness, also in relative skinfold thicknesses (Garn et al., 1988). However, in women (and men) who experience fluctuations in weight, relative skinfold thickness at the abdominal and iliac sites are the most responsive indicators of change in fatness (and it is of interest that women do not remodel fat at a rate different from that of men) (Garn et al., 1987). In both men and women, weight reduction has been found not to change WHR significantly, and fat distribution (android or gynoid) does not appear to change with weight loss (Andersen T. et al., 1989; Lanska et al., 1985a). However, significant correlations of WHR with BMI have been noted in both men (Ohlson et al., 1985) and women (Bengtsson et al., 1973; Lapidus et al., 1984), and this suggests that WHR is influenced by weight reduction and weight gain. In lieu of more consistent information on the interaction between total body fat and measurements of regional fat distribution, it would appear best to adjust measures of distribution by a valid estimate of either total or subcutaneous body fat and to report both sets of data. The BMI is not a valid enough index of total adiposity for this purpose.

Physical Activity

Physical activity may influence the distribution of body fat since since it can have a profound effect on total adiposity (Tremblay et al., 1985). It is therefore of interest that neither maximum oxygen consumption (on a graded treadmill exercise) nor total habitual caloric expenditure (based on a detailed physical activity questionnaire) has been found to be significantly correlated with WHR (adjusted for age and BMI) (Shimokata et al., 1989). This agrees with observations of the preceding section which noted no significant changes of WHR with alterations in weight and agrees with reports of relative consistency of body fat distribution. Aerobic exercise-training consistently and preferentially decreases abdominal fatness in *men* (Després et al., 1988a; Selby et al., 1990), but in women effects are inconsistent (Després et al., 1988a; Kaye et al., 1990).

Parity

Whether parity status is associated with WHR is currently unclear. A recent study which stratified for menopausal status found no significant difference between parous and nulliparous women and WHR; furthermore, this was observed in both pre- and

postmenopausal women (Tonkelaar et al., 1989). This lack of association has also been observed earlier in women not stratified for menopausal status (Edwards, 1950). But two studies have demonstrated a significant association of parity status with WHR (Lanska et al., 1985a; Lanska et al., 1985b; Kaye et al., 1990); both found a slight curvilinear relationship between WHR and number of pregnancies. Authors of both studies stress, however, that this association was likely due only to the very large sample populations utilized (52,953 women, and 40,980 women, respectively), stating that the magnitude of the effect was very small. Furthermore, they make the distinction of *biological* versus *statistical* significance, and suggest that this slight effect may be due to increased laxity of abdominal musculature with increasing parity.

Oral Contraceptives

A recent study has displayed no significant relationship between use of oral contraceptives and WHR in premenopausal women (Tonkelaar et al., 1989). Postmenopausal women studied retrospectively display no significant difference in WHR between "ever-" and "never-users" of oral contraceptives (Kaye et al., 1990). It would appear that oral contraceptive use does not influence WHR, but this area has not been studied extensively.

Alcohol Consumption

In men and women, some epidemiological studies indicate that high levels of alcohol consumption are associated with high WHR; this has been found by direct history as well as by registrations in alcohol temperance boards (Lapidus & Bengtsson, 1988; Björntorp, 1989; Troisi et al., 1990). This association appears independent of degree of obesity. Furthermore, chronic alcoholism is associated with pseudo-Cushing's syndrome, one characteristic of which is central adiposity (Proto et al., 1985). However, a negative association of WHR with alcohol consumption has been noted in postmenopausal women (Kaye et al., 1990), and no relationship between these two variables has been found in a sample of Mexican American and non-Hispanic white women (Haffner et al., 1986a). In 265 pairs of male twins ages 59-70 years, no relationship of WHR or subscapular/triceps ratio with alcohol consumption was found (Selby et al., 1990).

The Premenopausal Woman: A Special Case

It has long been known that premenopausal women are at lower risk than postmenopausal women and men in terms of developing complications of health. Risk for women has been shown to increase slightly with age, as also has WHR (Lapidus & Bengtsson, 1988), but virtually no study has stratified for menopausal status in investigating both health risk and adipose tissue distribution. There is now evidence indicating that: (a) premenopausal women specifically are at lower risk for certain complications of health; and (b) these women have a low WHR, reflecting gynoid distribution of adipose tissue (Tonkelaar et al., 1989). The association in premenopausal women of low relative health risk, low WHR and gynoid body fat distribution is not to be taken lightly. It ties in with the previous discussion regarding the positive association between health risk and WHR, high WHRs indicating android adiposity. Considering that the WHR is positively associated with health risk independent of degree of obesity, it follows that total body fat may be a less important indicator of health than the pattern of fat distribution, and that fat distribution on the trunk is more directly related to the health risks previously ascribed to obesity in general.

The best single statement about the special case of the premenopausal woman was made by Vague (1985):

> A woman has normally twice a man's fat mass, the mass of an obese man. As often obese as man is and fatter, she dies later and less often from obesity metabolic complications [*sic*]. Why the injustice? The answer: an obese woman is protected when she keeps her gynoid fat mass, an evidence of her child bearing nature. When her fat is android she dies like a man. (p. 6)

When one considers that men are predominantly of android body fat distribution there is an obvious implication: the female sex hormones may play a major regulatory role in gynoid fat distribution and, consequently, lack of predisposition to disease. There is considerable support for the latter part of this statement; the estrogenic dominance of premenopausal women appears to protect women from many complications of health, especially from cardiovascular disease, and postmenopausal women taking exogenous estrogens enjoy the same protection (Hazzard, 1986; Perlman et al., 1988; Hazzard, 1989; Barrett-Connor et al., 1989). More specifically, it appears that both adipose tissue distribution and associated health risk may be exacerbated by changes in relative androgenic/estrogenic balance.

At present, there is not widespread knowledge of a major regulatory role of the female sex steroids in the development and maintenance of gynoid adiposity. No comprehensive review is available in the literature. There is, however, considerable support for such a relationship, one which includes developmental, cellular and metabolic factors. Section I of Chapter 2 reviews and examines all the relevant literature on the association of female sex steroids with gynoid adiposity. The nature of the association of these two variables is fundamental to this thesis.

Assessment of Relative Androgenic/Estrogenic Activity

Methods

This area will be covered in greater detail in the following chapter. At this point it is sufficient to state that there are three main measurements which may be monitored and contrasted against either established norms or a control group in order to establish relative androgenic/estrogenic activity: (a) urinary (metabolized) sex hormone excretion; (b) plasma sex hormone levels; and (c) levels of sex hormone-binding globulin (SHBG). Measurement of the former present considerable difficulty due to fluctuating hormone levels, but the latter, its concentration being largely determined by the androgen to estrogen ratio, appears to be considerably more stable and may actually be a better measurement in certain situations.

Considerations

There are four main factors which must be considered in assessing relative androgenic and/or estrogenic activity. These are physical activity, parity, use of oral contraceptives and alcohol consumption.

Physical Activity

Physically active women display associations with amenorrhea, luteal phase suppression, menstrual dysfunction, altered endogenous sex steroid levels and low SHBG (Baker & Demers, 1988; Baker et al., 1981). Amenorrhea and menstrual dysfunction are associated with hormonal upsets and are easily screened from any investigation assessing hormonal balance. Rather, the possibility that physical activity may induce subtle changes in sex hormone levels and/or SHBG levels deserves consideration.

A recent review (Highet, 1989) provides evidence indicating that ovarian hormone excretion, by the pulsatile release of the pituitary gonadatrophic hormones follicle-

stimulating hormone (FSH) and luteinizing hormone (LH), which are in turn controlled by the hypothalamus via gonadotropin-releasing hormone (GnRH), may be disturbed by a variety of neural and hormonal inputs to the hypothalamus. It is stated that "even minor exercise will reduce the amplitude of leuteinising [*sic*] hormone-releasing hormone (LHRH) pulses which stimulate ovarian production of estrogen and progesterone (McArthur et al., 1980)." (Highet, 1989, pp. 92-93). This implies that any woman who exercises, even lightly, may be prone to alterations of sex steroid production. However, going directly to the primary source cited (McArthur et al., 1980b), it is obvious that the source does not substantiate the statement. The study concerned only three women; all were competitive athletes, and all were amenorrheic to begin with. Only *one* of the three displayed impaired LHRH release; consequently, the above quotation is a gross overstatement completely lacking any fundamental basis.

Other studies in the area have indicated reduced progesterone secretion and short luteal phase in regularly menstruating competitive athletes (Bonen et al., 1981; Loucks, 1990), but the effect of recreational athletic activity is unclear. As is the case with amenorrheic athletes, available evidence suggests that that it is the intensity of physical activity, moreso than the form it takes, which is associated with menstrual dysfunction (Highet, 1989). Thus competitive athletes, who by nature train at a high level of intensity, demonstrate associations with amenorrhea, oligo-ovulation, short luteal phase and low progesterone secretion, while persons engaged in recreational activity do not. Several studies have shown that untrained regularly menstruating women who gradually increased aerobic training intensity over long intervals (one year or more) display predominantly normal reproductive function (Boyden et al., 1983; Boyden et al., 1984; Bullen et al., 1984), and there is evidence indicating that SHBG is normal under conditions of physical activity, except in women with menstrual disorders (Ronkainen et al., 1985; Baker et al., 1981).

It seems reasonable to conclude that physical activity, unless intensely and excessively engaged in, does not affect sex hormone levels to any great extent. Any significant alteration would likely manifest itself as either amenorrhea or some form of menstrual dysfunction, and SHBG levels would almost certainly be altered. Mild and moderate forms of physical activity are inherently natural forms of human behavior; it seems inconceivable that anything other than a sedentary lifestyle would result in abnormal sex steroid production.

Parity

Several studies have found no consistent association between plasma and urine sex hormone levels and parity status (MacMahon et al., 1982b; Musey et al., 1987; Bernstein et al., 1985). A recent investigation utilizing vaginal cytology as an "inbuilt bioassay" also found no effect (Baron et al., 1988). Parity appears an essentially irrelevant variable with respect to androgenic/estrogenic balance.

Oral Contraceptives

Administration of oral contraceptive dosages of estrogens, natural or synthetic, clearly increases plasma levels of both estrogens and SHBG (Liukko et al., 1988; Venturoli et al., 1988; Key et al., 1989; Pugeat et al., 1988b; Swinkels et al., 1988). Levels of androgens have been shown to be lower also (Swinkels et al., 1988). In particular, it seems that SHBG is highly sensitive to oral estrogens (Pugeat et al., 1988b); there may also be down-regulation of SHBG which persists several years after use of oral contraceptives ceases (Key et al., 1989).

Alcohol Consumption

In men, chronic alcohol consumption inhibits secretion of LH and gonadal steroids; acute alcohol intake is associated with suppressed testosterone levels, and acute doses of alcohol may also have direct effects on gonadal production or secretion of testosterone (Mendelson et al., 1981). It is reasonable to assume that there are comparable effects in women, but evidence is limited.

In pre- and postmenopausal women, estrogen (Cauley et al., 1989) and progesterone (Björntorp, 1989) levels have been shown to decline with increasing (chronic) alcohol consumption. In pregnant women, chronic drinking (more than five drinks per week) is associated with decreased levels of both SHBG and testosterone (Ylikorkala et al., 1988). Acute effects on premenopausal women, however, appear to contrast sharply with the significant effects of acute alcohol intake in men. Studies have demonstrated no significant effect of alcohol on plasma levels of the pituitary gonadal hormones FSH and LH, nor on levels of the sex steroids estradiol, progesterone or testosterone (McNamee et al., 1979; Mendelson et al., 1981). While there may not be any acute effect of alcohol consumption on gonadal steroid levels in women, there certainly is a chronic effect. It might be prudent to take note of alcohol consumption in "normal" women undergoing sex hormone assays, especially consumption in excess of five drinks per week.

Effects of Smoking

It has been discussed that the nature of the adipose tissue distribution reflects associated health risks, android distribution clearly being associated with far greater risks than gynoid distribution. Considering that premenopausal women are protected from cardiovascular and some metabolic complications of health while retaining their gynoid fat mass, indicating estrogenic dominance, any phenomenon which increases relative androgenic/estrogenic activity might be expected to induce a shift towards android adiposity, thus incurring decreased protection and increased health risk. As will be discussed in Section II of Chapter 2, smoking is one phenomenon which increases relative androgenic/estrogenic activity in women, and there is evidence associating smoking with android adiposity (as indicated by greater WHR) in both men and women. These premises are also fundamental to the nature of this thesis, and the relevant literature will be reviewed and discussed shortly.

While smoking appears to be associated with android adiposity, there is ample evidence that both male and female smokers, as a group, weigh less for their height than nonsmokers, and that cessation of smoking leads to significant weight gain. More importantly, the inverse relationship of smoking with obesity has been described both cross-sectionally (Blitzer et al., 1977; Jacobs & Gottenborg, 1981; Gofin et al., 1982; Albanes et al., 1987; Kaye et al., 1990) and longitudinally (Blitzer et al., 1977; Lund-Larsen & Tretli, 1982; Garvey et al., 1974; Comstock & Stone, 1972; Stamford et al., 1986; Gordon et al., 1975), and it is clear that subcutaneous skinfold measurements and other indicators of body fatness are greater in nonsmokers than in smokers (Garn, 1985; Kromhout et al., 1988; Cox, 1989; Klesges et al., 1990). Utilizing the BMI as an index of obesity, and referring back to Figure 1-1, it is evident that smokers, with low BMI, are susceptible to respiratory diseases, digestive diseases and certain carcinomas. This is to be expected. But if one considers their greater WHR, smokers are at increased risk for development of diabetes, hypertension, heart attack and stroke, an association evident in Figure 1-2, where the relationship between BMI and cardiovascular disease was examined in isolation from other causes of mortality. The case of smokers clearly illustrates how a general measure of "overweight", or "underweight", in this case, is insufficient to account for overall health risk. The distribution of body fat must also be accounted for. Importantly, the strongest risk factor to emerge from prospective studies is the combination of low BMI and high WHR (Björntorp, 1988b), a clear description of smokers. The irony of smoking promoting both leanness (supposedly healthy) and android adiposity (increased health risk) ought not to be lost.

Summary and Conclusion

Obesity, as estimated by BMI, is clearly a crude measure, one which is generally inadequate as a valid predictor of overall health risk. Assessment of body fat distribution as indicated by WHR provides a better indication of health risk. Of the two main forms of adipose tissue distribution, android and gynoid, android adiposity is related to serious cardiovascular and metabolic complications of health; complications arising from gynoid adiposity are much less serious. Except in cases of extreme obesity, total body fat is a much lesser consideration than the distribution of body fat.

While men and postmenopausal women are generally of android distribution, premenopausal women predominantly display gynoid adiposity. Premenopausal women are also known to be at lower overall health risk, and it is thought that the estrogens are responsible for this protective effect. Premenopausal women display low WHRs due to gynoid adiposity, and low WHRs are associated with low health risk. That estrogenic dominance and low WHR are both associated with low health risk implies involvement of the estrogens in determining regional adiposity in premenopausal women. As will be discussed, there is considerable evidence for this contention. An anti-estrogenic and pro-androgenic effect of smoking in women will also be discussed, along with evidence associating smoking with high WHR. There is, however, no known mechanism for this effect of smoking on body fat topography, and there has never been reported an investigation of the effect of smoking on sex steroid levels and/or balance and measures of regional adiposity in premenopausal women.

Statement of the Problem

The purpose of this study is to investigate the nature of the interrelationship, if any, between smoking, sex steroid levels and balance and measurements of adipose tissue distribution in premenopausal women.

Importance and Relevance

The disposition of body fat in females appears to be, at least partially, under reproductive hormone control (Vague et al., 1985). As will be discussed, adipose tissue depots respond differentially according to gender, menopausal status and, especially, the

distinct demands of pregnancy and lactation. Realization of the important role of adipose tissue distribution and altered associations of health risk and morbidity with changes in this distribution indicate the need for greater understanding of the sexually dimorphic mechanisms responsible for distribution of the adipose tissue.

An association of smoking with increased androgenic relative to estrogenic activity and android adiposity within a single sample group would implicate a smoking-induced alteration of reproductive hormone levels or balance as a possible mechanism in the pathogenesis of android adiposity. Conversely, an association such as this would provide supporting evidence for the involvement of the female sex hormones in the development and maintenance of gynoid adiposity. Furthermore, the associated health risk of smoking would become even greater (than the presently known risks) for premenopausal women who smoke, and this risk is considerable even without the implied risks of android adiposity.

Women who smoke, compared to nonsmoking women, display greater risk and incidence of cardiovascular diseases (atherosclerosis, coronary heart disease -- acute myocardial infarction and chronic ischemic heart disease, angina pectoris, cerebrovascular disease, arteriosclerosis and venous thrombosis), cancer (lung, larynx, oral, esophageal, urinary bladder, kidney and pancreas) and non-neoplastic bronchopulmonary diseases (chronic bronchitis, pulmonary emphysema and chronic obstructive lung diseases), and maternal smoking during pregnancy is associated with numerous risks both to mother and child (U.S. Department of Health Education and Welfare, 1980). To this list might be added all the associated health risks of android adiposity (which do not bear repeating here). It is unclear whether the respective risk factors of smoking and android adiposity would merely be additive; there could be a synergistic effect involved. They might even be the same. But at the very least, the combined risks of smoking and android adiposity in women appear great indeed, and indicate the need for intervention. Considering the role female sex hormones appear to play in the development and maintenance of gynoid adiposity, an inverse association between estrogens, smoking and android adiposity -- or a positive association between androgens, smoking and android adiposity -- would have important implications for a mode of therapy or intervention aimed at decreasing overall health risk. At the same time, an association of smoking with altered hormonal profile and abnormal body fat distribution may serve to discourage smoking more than the known health risks, and there are surely some young female smokers who feel that the possibility of cardiovascular disease, pulmonary disorders and cancer are so remote as to be psychologically irrelevant. Along the same lines, such an association would also suggest that the decision to initiate smoking -- especially to control body weight -- is unwise.

In conclusion, it is acknowledged that cause and effect relationships cannot be proven with an epidemiologically-styled descriptive study. However, an investigation describing an inverse relationship between smoking, estrogenic relative to androgenic activity and measures of gynoid adiposity would suggest that these phenomena are not just incidentally interrelated, but that theirs is a causal relationship. Support for a causal relationship would include: (a) the results of an investigation describing such an interrelationship; (b) the supporting theoretical framework; and (c) agreement of differently designed studies indicating consistent associations between estrogens and gynoid adiposity, androgens and android adiposity, smoking and increased androgenic relative to estrogenic activity, and smoking and android adiposity.

Delimitations and Limitations

The sample group was composed of 56 subjects, this number exceeding that suggested by calculations of statistical power (Appendix A). All subjects were premenopausal females between the ages of 20 and 35 years characterized by normal cyclical menstrual history and no endocrine abnormality. Subjects who engaged in competitive, intense physical activity and/or more than eight hours of planned exercise per week were excluded. All subjects were from the greater Winnipeg area. Subjects were delimited to non-pregnant parous and nulliparous women who did not use oral contraceptives or any other estrogen-containing medication.

The sample group was split into two subgroups based on smoking status. Nonsmokers were delimited to women who had never smoked at all and/or former smokers who had (a) not smoked at all for at least the five year period preceding participation in the study and (b) not smoked for a period of time equal to or greater than the duration of the period for which they smoked. Smokers were delimited to women who had smoked a minimum of 8 cigarettes per day for at least five years.

Measures of regional adiposity were delimited to girths and girth ratios representative of relative adipose tissue distribution, and skinfold thicknesses at the biceps, triceps, subscapular, iliac crest, supraspinale, abdominal, front thigh and medial calf sites.

Measures of relative androgenic/estrogenic balance were delimited to assays for serum estrogen, testosterone and SHBG.

Limitations were imposed by delimiting the sample group to the above-stated parameters, with the implication of sample-specificity. However, the sample delimitations

are neither lax nor overly restrictive; they represent a reasonable compromise between internal and external validity.

No reasonable limitations result from delimiting measures of regional adiposity and relative androgenic/estrogenic balance to the stated parameters.

Hypothesis

Premenopausal female smokers will display greater androgenic relative to estrogenic balance or activity and greater measures of android adiposity than nonsmoking peers. An interactive effect of smoking with sex hormone balance and regional adiposity will be demonstrated.

Definition of Terms

Adipose Tissue

Tissue whose primary function is lipid storage (see Body Fat).

Adiposity

The amount of adipose tissue present in a given individual relative to his or her own age and stature.

Androgenic/Estrogenic Activity

Degree of androgenic activity *relative* to estrogenic activity.

Android Adiposity

Abdominal, central, centripetal, truncal, upper-body, or male-type localization of adipose tissue.

Body Fat

Tissue whose primary function is lipid storage (see Adipose Tissue).

Excess Physical Activity

Competitive athleticism and/or intense recreational activity and/or greater than eight hours of planned physical activity per week.

Fat

Ether extractable lipid (biochemical definition -- "fat" is not synonymous with "adipose tissue") (Ross W.D., 1989).

Free Androgen Index (FAI)

An estimate of "free" serum testosterone levels derived by dividing total serum testosterone by total serum SHBG (T/SHBG).

Free Estrogen Index (FEI)

An estimate of "free" serum estradiol levels, analogous to the FAI, derived by dividing total serum estradiol by total serum SHBG (E_2/SHBG).

Gynoid Adiposity

Gluteal-femoral, peripheral, centrifugal, lower-body, or female-type localization of adipose tissue.

Nonsmoker

A subject who has either never smoked at all, or a former smoker who has: (a) not smoked at all for at least the five year period preceding participation in the study; and (b) not smoked for a period of time equal to or greater than the duration of the period for which she smoked.

Obesity

Excess adiposity; implies value judgement about an "ideal" and some given departure from it.

Overweight

Excess body weight greater than some arbitrary standard defined in relation to height.

Proportional Sum of Central Skinfolds ($\propto SSF_c$)

Central sum of skinfolds (iliac crest, supraspinale and abdominal) adjusted for height, where height is considered to be an indicator of size, to quantify proportional differences in size.

Proportional Sum of Overall Skinfolds ($\propto SSF_o$)

Overall sum of skinfolds (biceps, triceps, subscapular, iliac crest, supraspinale, abdominal, front thigh, medial calf) adjusted for height, where height is considered to be an indicator of size, to quantify proportional differences in size.

Skinfold

A double thickness of skin and entrapped adipose tissue of a fold raised and encompassed with full tension on the pressure plates on a skinfold caliper applied to a specified site on the body (Ross W.D., 1989).

Smoker

A subject who has smoked a minimum of eight cigarettes per day for the five year period prior to participation in the study (Albanes et al., 1987).

Subject

A premenopausal female between the ages of 20 and 35 years who has met the selection criteria outlined in Chapter 3.

Assumptions

Girth Ratios

Perhaps the most fundamental assumption in any investigation utilizing a dimensionless ratio of circumferences -- such as the WHR -- to assess body fat distribution concerns the nature of the measurements themselves. What does a circumference really describe? The assumption is that it assesses outer (subcutaneous) adipose tissue and, particularly in the case of an abdominal circumference, internal adiposity. Thus, the WHR is supposed to assess fat distribution independent of total adiposity. But what of the factors which regulate the WHR -- are they the same in lean and obese individuals? This is a difficult question to answer, especially in view of the rather obvious speculation that muscular and skeletal factors may be more important determinants of WHR in lean individuals (Smith U., 1988). Furthermore, ratios may not be as simple or informative as they appear since the variance of a ratio is inexplicable from the contributions of variance of the numerator and denominator (Ross W.D., 1989). Thus, one fundamental assumption of the study was that girth ratios (such as the WHR) are valid methods by which to assess regional adipose tissue distribution.

Skinfold Measurements

Assessment of skinfolds require the following assumptions: (a) constant compressibility of tissue; (b) negligible skin thickness or skin thickness to be a constant fraction of the skinfold; and (c) fixed proportion of internal to external fat. None of these assumptions are actually true (Martin et al., 1985). Skinfolds, however, are a valid method by which to assess adiposity so long as illusions about individual predictions of percent fat are not entertained (Ross W.D. et al., 1988); this requires the further assumptions of fixed adipose tissue patterning and constant fat fraction of the adipose tissue in addition to the fundamental assumption that the human body is a two compartment model (fat and lean body mass compartments) with constant densities for each compartment, and none of these assumptions is true either (Martin et al., 1985).

Sex Hormone Assays

The fundamental assumption underlying sex hormone assays from a single sample of blood, even though removed at a standardized time of day at a standardized point of the menstrual cycle, is that the hormone levels assessed are representative of overall hormone levels. Sex hormone levels fluctuate considerably in women; apart from their rhythmic, or cyclic, release throughout the menstrual cycle, they also demonstrate pulsatile release throughout the day. Thus, levels could change over the course of just a few hours on any given day. Sex hormone-binding globulin demonstrates much less daily variability. In the hypothetically ideal (and therefore realistically impractical) situation, sex hormone levels could be assessed by removing and assaying blood every hour or half-hour over the course of a day, so as to obtain the *integral* of a plot of their levels over time. This integral would thus represent overall sex hormone levels, irrespective of daily fluctuation in levels and rates of production. The necessary assumption, therefore, is that a single sample of sex hormones on a specific day is representative of the integral of sex hormone levels over the course of this specific day. It follows, of course, that the integral of sex hormone levels over the course of one complete reproductive cycle (approximately one month) would be an even more ideal way by which to assess and contrast possible inherent differences in levels between groups.

CHAPTER 2: REVIEW OF LITERATURE

This chapter is organized into two main sections, each dealing with related, but yet distinct, areas which are fundamental to the stated problem. Each section is summarized at its conclusion, and the overall chapter is concluded with implications of the discussed material. Related literature is reviewed, examined and utilized to support the indicated hypothesis. Section I presents considerable evidence implicating involvement of the female sex hormones in the development and maintenance of gynoid body fat topography in women. It appears that were it not for the effects of reproductive hormones, women would display the android form of males. Observations of estrogen- (and progesterone-) deficient pre- and postmenopausal women support this statement. Specifically, sex- and age-dependent differences in adipose tissue distribution implicate involvement of the sex steroids, and changes in levels of these hormones at puberty and menopause relate to changes in adiposity in females at these times. Recent research at the cellular level has clearly illustrated regional differences in adipose tissue distribution as being mediated, at least in part, by the sex steroids directly. There have been shown to be definite metabolic differences between adipocytes of distinctly different anatomical regions, and changes of hormonal status have been shown to produce concomitant changes of metabolic and cellular characteristics of adipocytes in these differing anatomical regions. It appears that greater estrogenic relative to androgenic activity is an important factor in gynoid adiposity, just as greater androgenic relative to estrogenic activity is an important factor in android adiposity.

Section II deals with the direct effect of cigarette smoking in altering relative androgenic/estrogenic balance. In women, smoking appears to have an anti-estrogenic effect, and androgenic activity appears to be promoted. Either way, smoking increases androgenic relative to estrogenic activity. Possible mechanisms for this effect of smoking are discussed, as are effects on regional adiposity. Other hormonal effects of smoking which relate to androgen/estrogen balance are also considered, and effects of smoking on androgenic/estrogenic balance in men are discussed briefly.

The intentions of this review are: (a) to illustrate the involvement of the estrogens in promoting and maintaining a "healthy" gynoid adipose tissue distribution; and (b) to illustrate how smoking alters relative androgenic/estrogenic balance, resulting in an "unhealthy" android adipose tissue distribution.

Section I: Sex Hormones and Adipose Tissue Distribution

Sex- and Age-Dependent Differences in Regional Adiposity

As long ago as 1947 Vague reported that abdominally located fat was a typically male trait. A few years later he stated: "fat distribution is very definitely a sexual characteristic, but there is a high percentage of overlapping between one sex and the other, especially at the two extremes in life" (Vague, 1956, p. 24). In addition to discussing health risks of respective obesities Vague went further, stating that: (a) gynoid obesity was more frequent in women; and (b) android obesity more frequent in men, and interestingly, in menopausal women, where it was accompanied by other elements of virilization (Vague, 1956).

Other early researchers were also to note sex- and age- dependent differences in fat distribution. In 1953 Skerlj, Brozek and Hunt reported that with increasing age, the trunk gains more fat than the limbs, with men acquiring fat in the abdomen and women acquiring fat around the waist. Moreover, these researchers found that "younger women tend to be more massive in the legs while in older women the trunk gains soft tissue" (Skerlj et al., 1953, p. 592), resulting in "an increasing typological similarity of the sexes in late middle age" (Skerlj et al., 1953, p. 596). These observations essentially parallel those of Vague (1956).

The data of Edwards in 1951 show relatively greater values of subcutaneous fat thickness measurements above the waist in contrasting pubertal with menopausal women. In 1949 Angel, studying women, noted central fat deposition in preadolescent obesity and middle age, and contrasted with it the peripheral end of the continuum, fat localized in the limbs but not on the trunk.

An obvious question with respect to the above-noted observations of age- and sex-dependent differences in adipose tissue distribution concerns the period of life at which these dissimilarities become apparent. Are these differences always apparent? And, if not, then when do they become detectable?

Infancy and Childhood

According to Tanner (1978), subcutaneous fat begins to be laid down in the fetus at about 34 weeks gestational age, in the last trimester of pregnancy. But it has been reported that adipose tissue may develop as early as the 14-24th week of gestation (in the second trimester of pregnancy) (Poissonnet et al., 1984). Regardless, fat increases continuously to reach a peak approximately 9 months after birth in the average child (Tanner, 1978). Females have slightly more fat than males at birth (Tanner, 1978), but sexual dimorphism

of fat patterning is not apparent at this time (Poissonnet et al., 1984). Plotting skinfolds over time, Tanner (1978) has shown that skinfolds decrease between 9 months and 7 years, and then begin to rise again, with the sex difference increasing at about 8 years. Despite a prepubertal gain for both sexes, puberty results in truly radical differences becoming apparent between the sexes: skinfolds in males decrease, and skinfolds in females continue to increase (Garn & Clark, 1976; Tanner, 1978). Changes in skinfolds at different sites through childhood and adolescence are illustrated in Figure 2-1.

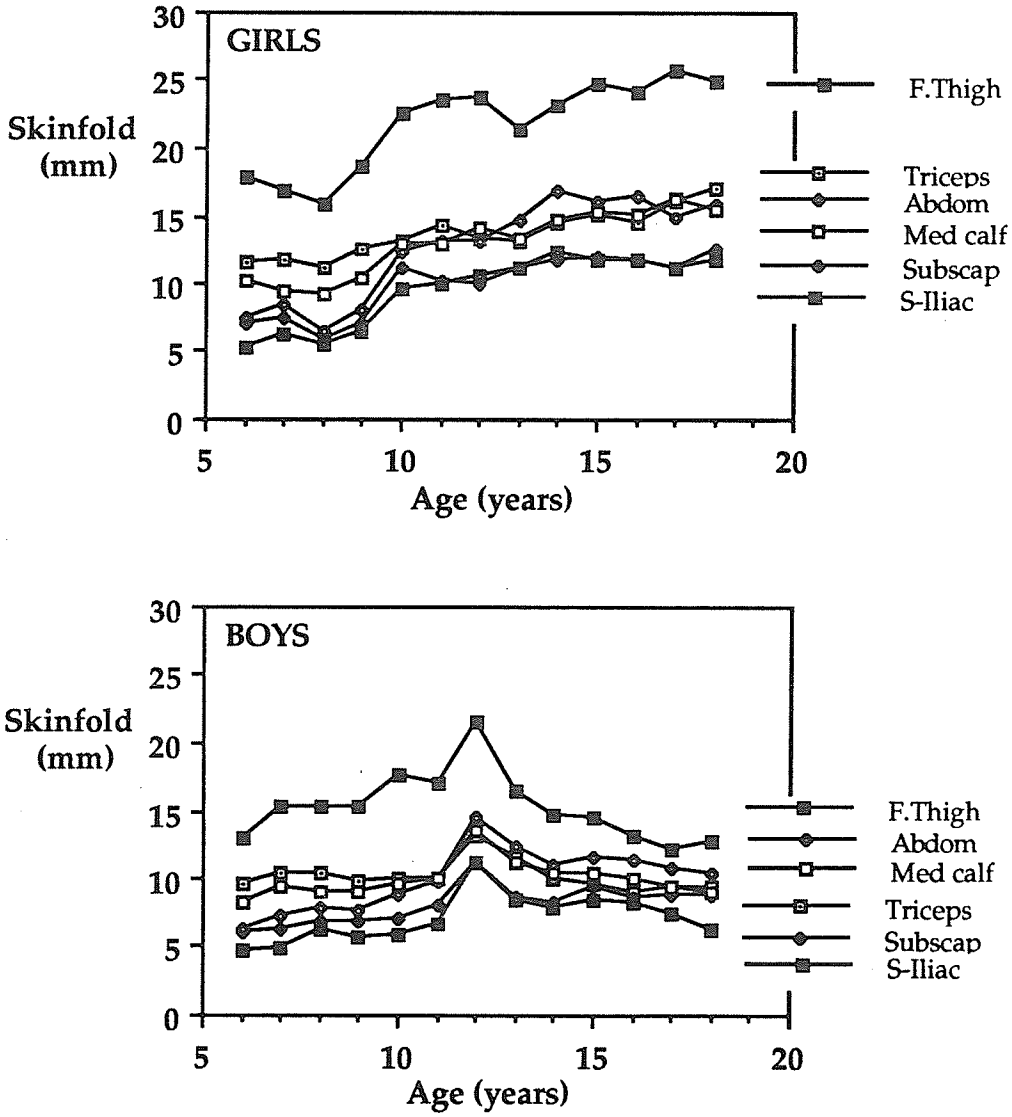

FIGURE 2-1. Age Changes in Skinfold Thickness at Six Sites. Cross-sectional data, adapted from Ross, W. D. & Marfell-Jones, M. J. (1982). Kinanthropometry. In S. D. McDougall, H. A. Wenger, & H. A. Green (Eds.), Physiological Testing of the Elite Athlete (pp. 75-115). Ottawa: Mutual.

Puberty

It is important to realize that the most major changes in the distribution of fat occur at the onset of adolescence as defined by the start of the growth spurt. The adolescent growth spurt begins at about 11 years for females and 13 years for males (Tanner, 1978); this two year difference makes it entirely inappropriate to compare fat distribution of males and females on the basis of age alone. The state of maturation is the crucial factor, not age.

It is difficult, however, to estimate fatness during rapid growth due to the differential growth rates of major tissues. The large increase in lean body mass and decrease in body fat in boys through puberty is illustrated by the fact that percent body fat (%BF) (by underwater weighing) can decline from about 21% at age 11 years to about 11% at age 18 years (Baumgartner et al., 1989) while skinfolds, despite a brief prepubertal peak (Figure 2-1), show little change (Baumgartner & Roche, 1988). For girls, %BF (about 26%) shows little change over the same age span (Baumgartner et al., 1989), whereas skinfold thicknesses increase significantly (Baumgartner & Roche, 1988).

But the above only illustrates that puberty results in quantifiable sex-dependent differences in the amount of adipose tissue. Of equal, if not more, importance are qualitative changes which occur at puberty (Figure 2-2). A two-decade follow-up study found that a clear sexual differentiation of body fat distribution appeared in the course of development: 85 out of 86 males (99%) were found to be of android fat distribution, and 50 out of 78 females (64%) were found to be of the gynoid type, based on various skinfold ratio indices which discriminated central versus peripheral body fat distribution (Rolland-Cachera et al., 1989; Rolland-Cachera et al., 1990). Also contributing to development of the gynoid distribution in pubertal girls is a rapid increase in thigh and calf skinfold thicknesses with no concomitant rate of increase at upper body sites (Tanner, 1962), a phenomenon evidenced also by changes in the waist-to-thigh girth ratio (WTR) (Figure 2-3). Several other studies have found an android pattern to develop in boys but few girls at adolescence (Harasha et al., 1980; Mueller, 1982; Baumgartner et al., 1987), and in a study of the stability of fat patterns across four sex and ethnic groups, a trunk-extremity contrast was the only fat pattern homogeneous across all four groups, accounting for 34 to 57% of the total variance in subcutaneous adipose tissue distribution (Baumgartner et al., 1986). A centripetal distribution developed in males, but not females, during adolescence (Baumgartner et al., 1986). The major point to be made here is qualitative, not quantitative: adolescence results in female adipose tissue becoming more general in distribution, tending toward the periphery in contrast to males, who tend to localize fat centrally.

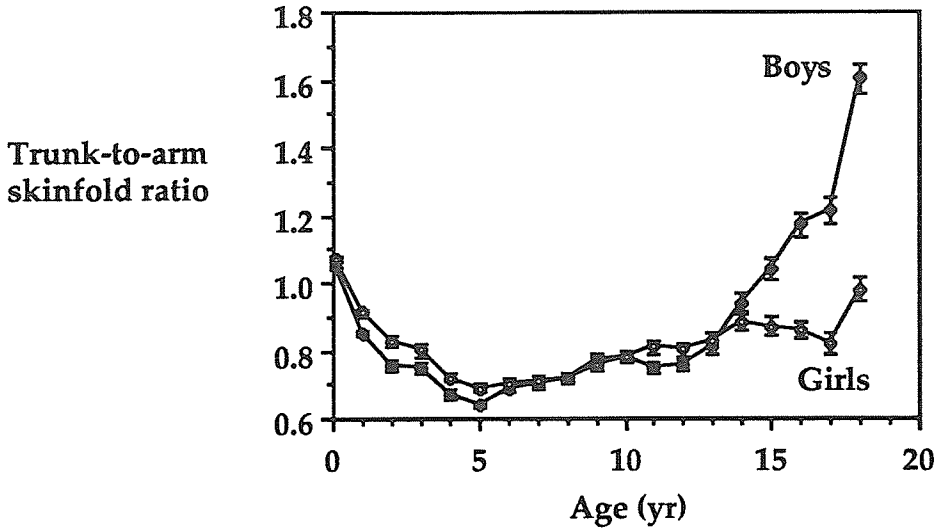

FIGURE 2-2. Pubertal Changes in Body Fat Distribution. Trunk-to-arm skinfold ratio (subscapular + suprailiac/ biceps + triceps) from birth to maturity in boys and girls, showing means and standard errors. Longitudinal data, adapted from Rolland-Cachera, M.-F., Bellisle, F., Deheeger, M., Pequignot, F. & Sempe, M. (1990). Influence of body fat distribution during childhood on body fat distribution in adulthood: a two-decade follow-up study. International Journal of Obesity, 14, 473-481.

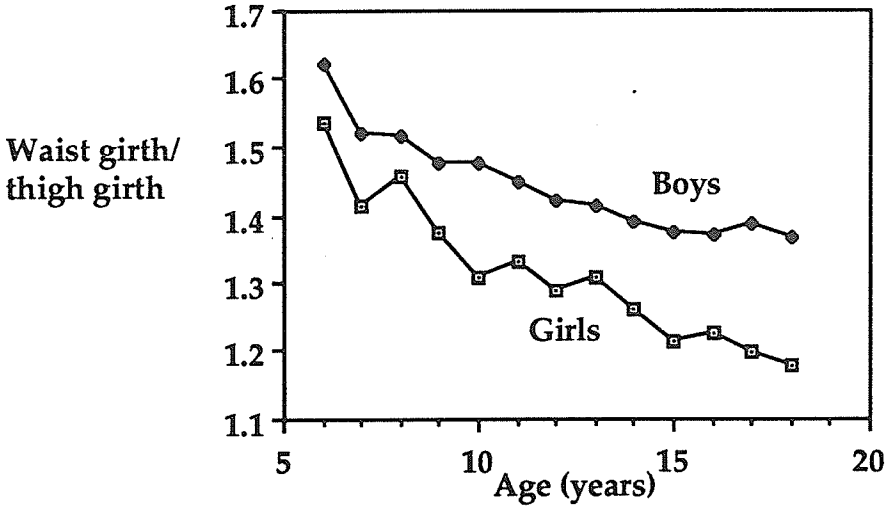

FIGURE 2-3. Age Changes in Waist-to-Thigh Girth Ratio. Cross-sectional data, adapted from Ross, W. D. & Marfell-Jones, M. J. (1982). Kinanthropometry. In S. D. McDougall, H. A. Wenger, & H. A. Green (Eds.), Physiological Testing of the Elite Athlete (pp. 75-115). Ottawa: Mutual.

It is thus obvious that qualitative changes in fat distribution at puberty are reflected by quantifiable changes among the sexes; there becomes sexual dimorphism in terms of both the amount and distribution of body fat. But it is important to appreciate that neither type of fat distribution is confined solely to one sex or the other; a relatively small percentage of females display the android distribution, and a still smaller percentage of males display the gynoid form (Vague et al., 1969). Obese adolescents, in particular, appear not to display the sexual dimorphism (with respect to fat patterning) characteristic of the non-obese (Katch et al., 1989; Parízkova et al., 1989), and it has been stated that obesity which has its origins during or close to adolescence may be characterized as an androgenous type of obesity (Deutsh et al., 1985).

Menopause

In men, the degree of android adiposity tends to increase with age as fat is deposited centrally. Women tend to accumulate fat as they age, but still retain the gynoid pattern. However, around menopause, women appear to undergo a shift from gynoid to android fat distribution, reducing sexual dimorphism. This phenomenon has been noted frequently (Angel, 1949; Edwards, 1951; Skerlj et al., 1953; Vague, 1956; Chien et al., 1975; Vague et al., 1978; Frisancho & Flegel, 1982; Rutishauser & McKay, 1986). Of further interest, and in support of the above, is the observation of a significant shift in somatotype in females after age 40; women become less ectomorphic and more meso-endomorphic (Bailey et al., 1982).

It is difficult to estimate the magnitude of this menopause-associated shift from gynoid to android adiposity in women. To be sure, the shift seems to coincide with the upper limit of reproductive age -- just as the lower limit of reproductive age seems to coincide with the onset of gynoid adiposity in girls -- but there is a confounding factor. Overall fatness tends to increase with age, and BMI has been found to correlate with WHR in both men (Ohlson et al., 1985) and women (Lapidus et al., 1984). Increases of overall fatness with advancing age -- especially prior to menopause -- might "smooth out" the increase in android adiposity in women at menopause. This possibility is illustrated by Figure 2-4: changes in WHR begin at about 35 years of age, substantially before menopause, which generally occurs at about 52 years of age in North America (C. Faiman, personal communication, August 14, 1990). There is, however, another shift in WHR at about 46 years of age, and it is hypothesized that if overall fatness did not change with age, the increase in android adiposity at menopause might be more dramatic.

In summary, there are relatively few differences between males and females until puberty in terms of adiposity. In girls, puberty results in essentially no change in relative

(total) adiposity, but fat distribution tends to become gynoid. In boys, total adiposity decreases markedly, and fat distribution becomes android. These aspects of sexual dimorphism are maintained until menopause, when women undergo a shift from gynoid to android fat distribution. As Vague has pointed out, though fat distribution is very definitely a sexual characteristic, there is a high degree of overlap between one sex and the other, especially at the two extremes in life (Vague, 1956). This suggests that hormonal changes coinciding with puberty and menopause are connected to the pattern of adipose tissue distribution in humans.

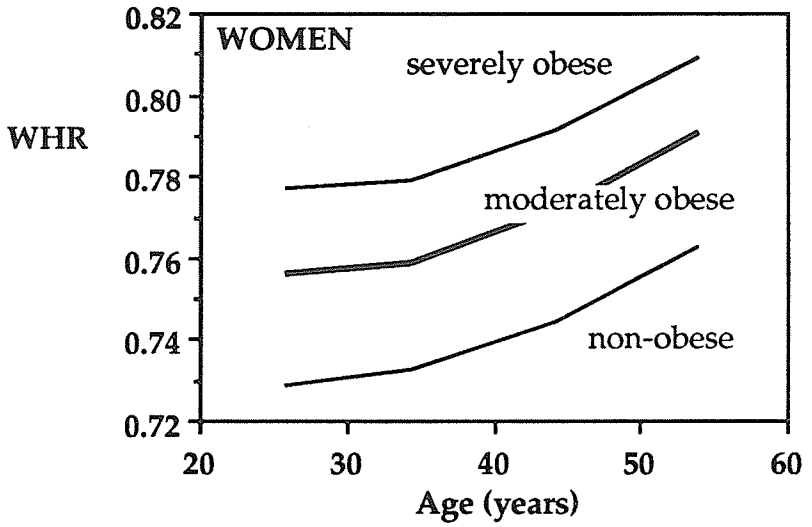

FIGURE 2-4. Changes in Waist-to-Hip Girth Ratio with Age in Women. Adapted from Lanska, D. J., Lanska, M. J., Hartz, A. J., Kalkhoff, R. K., Rupley, D. & Rimm, A. A. (1985). A prospective study of body fat distribution and weight loss. International Journal of Obesity, 9, 241-246.

Female Sex Hormones

Physiology

The female sex hormones are the estrogens and progestins. As gonadal steroid hormones, they act through a nuclear mechanism similar to that employed by the adrenal steroid hormones (Granner, 1988). The estrogens are a family of hormones produced in ovarian and extraovarian tissues. Of the ovarian estrogens, the most active and important naturally occurring hormone is estradiol (17ß-estradiol); estrone and estriol are also produced, but their biological activity is less than that of estradiol (Best & Taylor, 1985). As will be shown, a substantial amount of estrone is also of extraovarian origin. This review will focus mainly on estradiol (E_2) and estrone (E_1); levels of estriol (E_3) are relatively low in non-pregnant women. However, during pregnancy, estriol becomes the major estrogen, accounting for 80-95% of total estrogen synthesis in late pregnancy; it

begins to be produced by the placenta at about the ninth week of pregnancy, as ovarian steroid production declines (Best & Taylor, 1985). Progesterone is the most active of the progestins; it is produced and secreted by the corpus luteum (Granner, 1988).

Estrogens are formed by the aromatization of androgens (dehydroepiandrosterone -- DHEA) in a complex process which involves 3 hydroxylation steps, each of which require oxygen (O_2) and nicotinamide adenine dinucleotide phosphate (NADPH) (reduced). The aromatase enzyme complex includes a cytochrome P-450 mixed-function-oxidase (Granner, 1988). Overall pathways of estrogen production and metabolism are illustrated in Figure 2-5. Estrone is formed if the substrate of the aromatase enzyme complex is androstenedione (via aromatization), whereas estradiol results if the substrate is testosterone. However, estradiol may also result from estrone. Estrone is therefore a linchpin in a series of reversible and irreversible reactions, and a precursor for a series of irreversible reactions via: (a) the 16α pathway, leading to 16α-hydroxyestrone and then estriol; and (b) the alternate hydroxylation at C-2, which yields the catechol estrogens (Hershcopf & Bradlow, 1987). These two reactions are mutually exclusive and of physiologic significance in that the products of these two pathways have distinctly different biological properties (Fishman & Martucci, 1980). The two catechol estrogens (hydroxyestrogens), primarily 2-hydroxyestrone and 2-methoxyestrogen, are the major metabolites of estrogen in all mammalian species; they have weak estrogenic activity but are potent agents in the central nervous system (where they are also found) (Granner, 1988). Estrone and its products, 16α-hydroxyestrone and estriol, display relatively more activity (Fishman & Martucci, 1980), but a substantial amount of 16α-hydroxyestrone is not reduced to estriol (Hershcopf & Bradlow, 1987).

While in normal young women most circulating estradiol is ovarian in origin, some estradiol may be provided by the peripheral (extraovarian) aromatization of testosterone, but this extraglandular contribution is considered to be of little significance (Enriori & Reforzo-Membrives, 1984). Exempting rare cases of cortical tumors, it has never been shown that the adrenal cortex can directly produce and secrete estradiol (Enriori & Reforzo-Membrives, 1984).

Estrone is also of ovarian origin in young women, although not dominantly so. Studies have shown that up to 50% of the daily production of estrone is produced by peripheral aromatization of androstenedione in premenopausal women (Granner, 1988; Siiteri & MacDonald, 1973). A third source of estrone is the extraglandular oxidation of estradiol (Enriori & Reforzo-Membrives, 1984); estradiol is metabolized principally via estrone (Fishman et al., 1960; Gurpide et al., 1962).

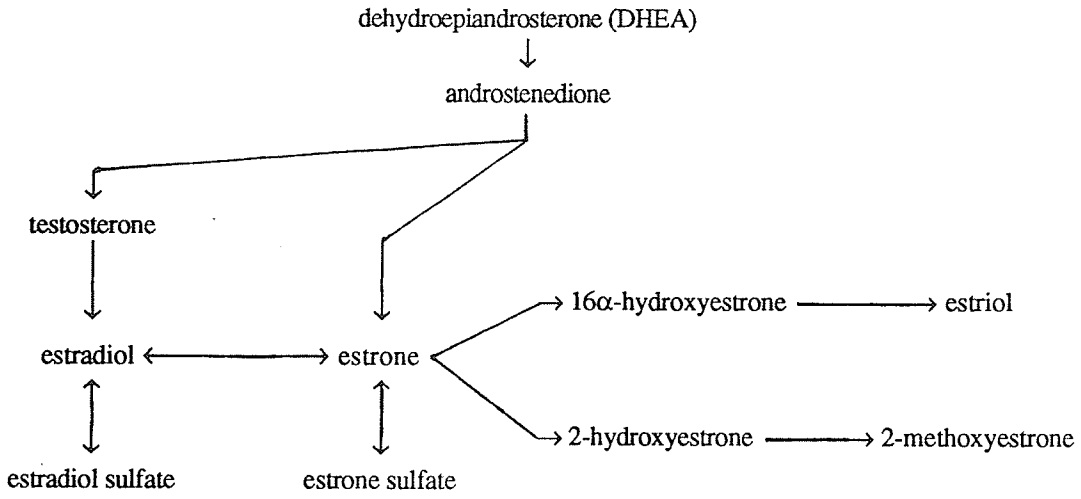

FIGURE 2-5. Pathways of Estrogen Production and Metabolism. Adapted from Hershcopf, R. J., & Bradlow, H. L. (1987). Obesity, diet, endogenous estrogens, and the risk of hormone-sensitive cancer. American Journal of Clinical Nutrition, 45, 283-289.

Thus, in the normal young female, significant amounts of estrogens are produced by peripheral aromatization of androgens. In males, peripheral aromatization is also an important pathway, accounting for 80% of the production rate of estradiol from testosterone (Granner, 1988).

The rate of secretion of ovarian steroid hormones varies throughout the menstrual cycle and is directly related to the rate of synthesis in the ovary (Granner, 1988). These compounds are not stored; they are secreted when they are produced.

All intermediates in the biosynthesis of estrogens are secreted in varying amounts. According to Best and Taylor (1985): in the early follicular phase, testosterone is secreted at a higher rate (260μg/day) than estrogens (60μg/day). By the late follicular phase, estrogen secretion has surpassed that of testosterone, reaching a secretion rate of 400-900μg/day. During the luteal phase, approximately 300μg/day of estrogen are secreted. At that time, progesterone is secreted in milligram amounts (25mg/day), surpassing all other ovarian steroids. Table 2-1 provides normal plasma values of major estrogens and androgens in men and women.

Estrogens and progestins are bound in varying degrees to plasma transport proteins. About 98% of plasma estradiol and estrone is tightly bound to sex hormone-binding globulin (SHBG); some is also more loosely bound to albumin (Bruning, 1987; Moore et al., 1987). Non-protein bound or "free" estradiol is considered to be more

TABLE 2-1. Normal Values of Major Plasma Estrogens and Androgens in Men and Women (nmol/L). Adapted from Eldrup, E., Lindholm, J., & Winkel, P. (1987). Plasma sex hormones and ischemic heart disease. Clinical Biochemistry, 20(2), 107.

	Estradiol	Estrone	Testosterone	Androstenedione
Premenopause				
Follicular Phase	0.13-1.46	0.18-0.74		
Luteal Phase	0.37-0.73	0.26-0.37		
on average			0.69-2.08[1]	1.70-10.50[2]
Postmenopause	0.03-0.10	0.11-0.22	0.50-1.70	1.70-3.10
Men	0.04-0.15	0.17-0.40	7.00-38.00	1.70-5.24

[1]Testosterone levels are ~ 20% higher at midcycle
[2]Androstenedione levels are ~ 15% higher at midcycle
Conversion factors from nmol/L to pg/mL: Estradiol: 273.37; Estrone: 270.36; Testosterone: 288.41; Androstenedione: 286.40

available for biological activity than protein-bound estradiol (Bruning, 1987), but estradiol bound to albumin may also be biologically active (Moore et al., 1987). Sex hormone-binding globulin binds to estradiol about 5 times less avidly than it binds to testosterone (Granner, 1988). Estriol exhibits minimal SHBG activity (as is also the case with the more abundant 16α pathway product, 16α-hydroxyestrone) (Hershcopf & Bradlow, 1987). As will be discussed, levels of SHBG are particularly important with respect to fat distribution. Progestins bind to corticosteroid-binding globulin (CBG), which has little affinity for estradiol, and even less for testosterone, or estrone (Granner, 1988).

Childhood and Puberty

Apart from transient increases in estrogen production in female infants shortly after birth, serum levels of both gonadotropins and sex steroids return to basal levels several months after birth (Winter, 1978). There is, however, evidence of continuing gonadotropin-mediated estrogen secretion in girls (Ross G.T., 1974), but sex steroids have not been shown to have any role in growth before puberty.

In girls there is an initial increase in serum follicle stimulating hormone (FSH) and luteinizing hormone (LH) at about 8 years; this is followed by an increase in ovarian estrogen secretion at about age 11, which coincides roughly with the appearance of labial hair or of a subareolar breast bud (Faiman & Winter, 1974). These are the first physical signs of puberty, often referred to as (Tanner) Stage 2. Gonadotropin and estrogen levels

continue to rise, albeit in a very erratic and roughly rhythmic manner (Winter & Faiman, 1973), as height velocity increases, the secondary sex characteristics develop and skeletal maturation occurs (Tanner, 1978). Menarche occurs sometime in mid-puberty at about Stage 4, with development of adult breast, pubic and axillary hair. Height velocity has peaked and is decreasing when menarche occurs. It ought to be clear that the pubertal rise in estrogens precedes menarche; this is important when considering studies attempting to relate point of maturation as indicated by menarche to changes in fat patterning. The relevance of maturational timing and its effect on fat patterning will be discussed shortly.

Menarche is induced when there is a sufficient decrement in estradiol levels during one of the roughly rhythmic fluctuations; this allows the first withdrawal bleeding to occur (Winter, 1978). Estradiol levels continue to fluctuate erratically after menarche and may not be associated with ovulation, but an adult menstrual rhythm appears 6 to 9 months post-menarche (Faiman & Winter, 1974). Thus, since progesterone production requires FSH-mediated follicular estradiol production to trigger a mid-cycle ovulatory surge of LH and FSH, a sustained luteal rise in serum progesterone levels may not occur until several months after menarche.

Concomitant with increasing gonadal secretion of sex steroids at puberty is increased secretion of the adrenal androgens dehydroepiandrosterone and androstenedione (Winter, 1978). While the adrenal androgens play a significant role in girls in the adolescent growth spurt and growth of sexual hair, their influence in boys is confounded by the more dramatic effect of gonadal testosterone (Winter, 1978). It should be noted that the weak adrenal androgens can be converted peripherally to stronger androgens (mainly testosterone) as well as estrogens (via aromatization) (Granner, 1988).

The onset of puberty and rise in sex hormone levels represent one of the most important periods in a young girl's life. Puberty signifies the onset of the adolescent growth spurt, development of secondary sex characteristics and skeletal maturation. It is the time of greatest sex differentiation since the early intra-uterine months, and gives rise to the sexual dimorphism characteristic of adult males and females. Fat patterning is an aspect of sexual dimorphism and it appears likely that the surge of female sex hormones at puberty is in some way involved with this patterning.

Menopause

In postmenopausal women, ovarian estrogen production ceases almost completely, although androgens continue to be produced by the ovaries (Judd et al., 1974; Greenblatt et al., 1976). Testosterone production is hardly decreased (in comparison to the premenopausal years), but the postmenopausal ovaries secrete only minor amounts of

androstenedione and DHEA (Vermeulen, 1983). Levels of plasma estradiol and estrone are lower than those observed at the follicular phase of the normal menstrual cycle in young women (Samoljik et al., 1977), although estrogenic cervical smears have been shown to persist postmenopausally in obese women (De Ward, 1969). According to a recent review (Enriori & Reforzo-Membrives, 1984), levels of estrone are greater than estradiol by approximately 2:1 or 3:1 (Judd et al., 1974; Samoljik et al., 1977; Vermeulen & Verdonck, 1978). Men have comparable estrogen level ratios (Samoljik et al., 1977). In the follicular phase of normal young women, circulating levels of estradiol are equal to or greater than those of estrone; thus, the relationship estrone/estradiol (E_1/E_2) is lower, oscillating around the unit or even less (Samoljik et al., 1977; Santen et al., 1978). After menopause, serum concentrations of both estrone and estradiol have been found to stay constant with increasing age (Jensen et al., 1985b).

As reviewed (Enriori & Reforzo-Membrives, 1984), a postmenopausal ovarian source of estrone is not likely considering that: (a) the concentration of estrone and the relationship estrone/androstenedione (E_1/A) in castrate women is similar to values found in normal postmenopausal women (Barlow et al., 1969; Saez et al., 1972; Vermeulen, 1976); and (b) estrone levels in the plasma of the ovarian vein are similar to those of peripheral vein blood (Judd et al., 1974). Several studies have shown that the adrenal cortex is not directly responsible for the secretion of estrone (Baird et al., 1969; Greenblatt et al., 1976), although this could account for up to 10% of circulating estrone (Baird et al., 1969). But the final evidence is that values for the production rate of estrone derived from androstenedione and total estrone production rate are almost identical in postmenopausal women (45.4μg vs 46.3μg per 24 hours, respectively) (Siiteri & MacDonald, 1973); it should be noted that the rate of peripheral aromatization of androstenedione to estrone increases at the menopause as ovarian production declines (Jensen et al., 1985b). Therefore, in the postmenopausal woman, it can be stated that ovarian or adrenal production of estrogen is essentially irrelevant; the main source is via peripheral aromatization of androstenedione to estrone.

Regarding the synthesis of estradiol in postmenopausal women (and discounting direct secretion by the adrenal cortex) there are two possibilities concerning its extraglandular production: either the aromatization of testosterone or the reduction of estrone. The latter explanation is considered the most important (Enriori & Reforzo-Membrives, 1984; Soules & Bremner, 1982) for the following reasons: (a) in postmenopausal women testosterone is very inefficiently aromatized to estradiol, and the production rate of testosterone is low (250μg/day) (Longcope et al., 1969); (b) androstenedione is available in much greater quantities (1.0 - 3.0 mg/day), and is much

more efficiently converted to estrone (1.3% or more vs 0.07% for aromatization of testosterone to estradiol) (Siiteri & MacDonald, 1973; Longcope et al., 1969); (c) aromatization of androstenedione in human adipose tissue is greater than that of testosterone both *in vivo* (Longcope et al., 1978) and *in vitro* (Forney et al., 1981); (d) circulating levels of androstenedione are considerably greater than those of circulating testosterone (Judd et al., 1974; Vermeulen & Verdonck, 1978); and (e) the conversion rate of estrone to estradiol is high, around 6.5% (Longcope et al., 1968). As with the rate of peripheral aromatization of androstenedione to estrone, the rate of conversion of estrone to estradiol also increases at the menopause (Jensen et al., 1985b). Dehydroepiandrosterone (DHEA) and its sulfate ester (DHEA-S), being the most abundant androgens which circulate in human plasma, could theoretically be considered important precursors of estradiol; this is not the case, however, since they have a low coefficient of conversion to estrogens (MacDonald et al., 1976). Therefore it may be stated that estrone appears to be the main precursor for the extragonadal biosynthesis of estradiol.

Briefly, a discussion of the role of conjugated estrogens in postmenopausal women is in order. Conjugated estrogens are those which are substrates for hepatic enzymes which add glucuronide or sulfate moieties (Granner, 1988). Conjugated estrogens are water soluble and of diminished biological activity; they are not bound to transport proteins (Best & Taylor, 1985). Conjugation increases solubility in an aqueous medium, thus, these estrogens are readily excreted in the urine, bile and feces (Best & Taylor, 1985; Granner, 1988). It has been demonstrated in cattle, using 14C-labelled estradiol, that 17ß-estradiol and estrone are the major metabolites in both muscle and adipose tissue, while the conjugated estrogens are the major metabolites in the liver and kidney (Kaltenbach et al., 1976).

The conjugated estrogens are estrone sulfate (E_1S) and estradiol sulfate (E_2S). Estrone sulfate is the principal estrogen in the blood of men and premenopausal women (Wright et al., 1978; Nunez et al., 1977; Carlström & Sköldefors, 1977; Hawkins & Oakey, 1974). Changes of estrone and estradiol throughout the menstrual cycle are accompanied by parallel changes in their corresponding sulfates (Nunez et al., 1977). Levels of E_1S are 10 to 15 times higher than those of nonconjugated E_1, while the concentration of E_2S is two to four times lower than that of nonconjugated E_2; the concentration of E_1S surpasses by two to three times the addition of the remaining estrogens (Nunez et al., 1977).

At postmenopause estrone sulfate (E_1S) is the most abundant plasma estrogen (Roberts et al., 1980); most E_1S and estradiol sulfate (E_2S) is peripherally synthesized from non-conjugated estrone and estradiol (Ruder et al., 1972). The liver is the most active

organ for sulfation; sulfation is directed in favour of E_1S (Longcope & Williams, 1974). While it is possible that conversion of estradiol to estrone as well as sulfation of estrone may be mechanisms of the estrogen effectors designated to facilitate the intracellular transformation of E_2 into E_1, thus favoring the elimination of the latter as sulfate due to its increased solubility, E_1S ought not to be considered exclusively as a metabolite for excretion (Enriori & Reforzo-Membrives, 1984). E_1S is easily hydrolyzed to estrone and estradiol (Payne et al., 1973), and in plasma E_1S circulates bonded to proteins (Rosenthal et al., 1972), suggesting that E_1S might be considered an inactive plasma reservoir of potentially active estrogens (Enriori & Reforzo-Membrives, 1984).

Up until this point neither the tissues involved in peripheral aromatization of androgens to estrogens nor their respective participation in this occurrence have been noted. Aromatization in humans and experimental animals may occur in the liver (Smuk & Schwers, 1977), kidney (Frieden et al., 1968), isolated human hairs (Schweikert et al., 1975), human fatty marrow (Frisch et al., 1980), cultured human fibroblast (Schweikert et al., 1976), central neuroendocrine tissues (Naftolin et al., 1975), rat bone (Vittek et al., 1974) and human muscle (Longcope et al., 1978), but by far the most important location in humans is adipose tissue (Bolt & Göbel, 1972; Schindler et al., 1972; Siiteri & MacDonald, 1973; Longcope et al., 1978; Forney et al., 1981; Grodin et al., 1973; Nimrod & Ryan, 1975; Riskallah et al., 1975; Longcope et al., 1976; Perel & Killinger, 1979; Longcope et al., 1982).

Lastly, certain tumors (Wotiz et al., 1955; Kirschner et al., 1974), especially some types of breast cancer (Miller et al., 1974; Perel et al., 1981), are also capable of aromatizing androgens. In these cases, locally produced estrogens could have a marked influence on the tumor, modifying its rate of growth even if unable to take up circulating estrogens; this mechanism might provide sufficient intratumoral estrogens without altering corresponding plasma levels (Enriori & Reforzo-Membrives, 1984). Irrespective of tumors, the local influence of estrogens within peripheral tissue, especially adipose tissue, could play a role in certain special functions like the neuroendocrine control of differentiation (Enriori & Reforzo-Membrives, 1984). Even if the amount of aromatization is very low, the potency of estrogen for biological action is very high, often 10 to 100 times higher than that of the parent androgen (Enriori & Reforzo-Membrives, 1984). As will be discussed, estrogen is a highly potent catalyst of adipocyte differentiation both at puberty and throughout adult life.

Sex Hormone-Binding Globulin

It has already been discussed that the estrogens are transported bound to SHBG and the significance of SHBG with respect to adiposity was hinted at. Because this binding globulin plays a substantial role in mediating the effect of sex hormones on adiposity, further elaboration is warranted.

There are sex- and age- dependent differences in levels of SHBG. Levels of SHBG are greater in prepubertal boys and girls than in adult men, and similar to the levels of normal adult women (Egloff et al., 1981; Duignan, 1976; Rosenfield, 1971). Concentrations in women are generally twice those of men (Egloff et al., 1981; Rosenfield, 1971). At puberty, SHBG decreases significantly in boys while levels stay relatively constant or increase slightly in girls; after menopause, SHBG decreases to values similar to those of adult men (Murayama et al., 1978; Duignan, 1976). Sex hormone-binding globulin increases substantially in men over 50 years of age (Vermeulen & Verdonck, 1972; Duerr & Pirke, 1973).

Values of SHBG are attained during pregnancy which possibly are never attained by any other physiologic or pathologic condition (Duignan, 1976; Vermeulen & Verdonck, 1972). Estrogen administration increases SHBG levels (Fex et al., 1981; Ruder et al., 1971) while androgen and somatotropin administration decreases them (Ruder et al., 1971; Vermeulen & Verdonck, 1972). These and other numerous observations of the interrelationship between SHBG and relative androgenic/estrogenic balance suggest that an increase in SHBG represents increased estrogenic activity, or a "feminizing" factor, while a decrease in SHBG represents increased androgenic activity, or a "masculinizing" factor (Peiris et al., 1989; Evans et al., 1983; Vermeulen et al., 1969; Enriori & Reforzo-Membrives, 1984). Since testosterone levels after menopause are either comparable to those of young women (Vermeulen & Verdonck, 1978) or else tend to increase slightly (Chakravarti et al., 1976), the postmenopausal decrease of SHBG could be due to either the decrease in synthesis of estradiol, or an increase of testosterone over the years post-menopause, or both (Enriori & Reforzo-Membrives, 1984). Regardless, the testosterone/estradiol (T/E_2) ratio would be increased, thus explaining the "masculinizing" signs frequently observed post-menopause along with low SHBG (Enriori & Reforzo-Membrives, 1984).

Because (a) the concentration of SHBG is largely determined by the androgen to estrogen ratio (Peiris et al., 1989), and (b) plasma values of SHBG are not significantly modified throughout the menstrual cycle in spite of great changes in levels of circulating estrogens (Wu et al., 1976), it appears that this binding globulin is a valid marker of relative androgenic/estrogenic activity. Thus it has been suggested, based on inconsistent

or insignificant results of urinary metabolite excretion and plasma estrogen level measurements (Bruning, 1987), that (a) SHBG levels may reflect tissue sensitivity and the impact of exposure to fluctuating levels of sex hormones for several days or more, and (b) measurement of SHBG may provide a better indication of sex steroid activity than a single sample of circulating hormones (Stefanick et al., 1987).

Sex Hormones in Relation to Regional Adiposity

On the average, adipose tissue represents from about 10% in the lean to 50% of body weight in extremely obese persons; this fat mass corresponds to about 3×10^{10} cells, mainly adipocytes (Björntorp & Martinsson, 1966). Roughly 10% of an average adipocyte is cytoplasm, 90% is fat, and the cell membrane and cytoplasm of an adipocyte are stretched as a narrow rim around the fat globule. In theory, this large surface area should favour uptake of steroid hormones, and this is indeed the case. Many findings provide evidence that adipose tissues are *bona fide* target tissues for sex steroids. In experimental animals, cytoplasmic estrogen and progestin receptors have been demonstrated in adipose tissue (Wade & Gray, 1978; Gray & Wade, 1979; Gray & Wade, 1980; Gray & Wade, 1981; Gray et al., 1981; Wade et al., 1985), but in *human* adipose tissue, however, different techniques such as gel filtration chromatography, isoelectric focusing and monoclonal antibodies have failed to demonstrate any cytoplasmic estrogen and progestin receptors (Rebuffé-Scrive, 1988). Further experiments are being conducted in order to determine the presence of nuclear estrogen and progesterone receptors in human adipose tissue. Regardless, one might expect that plasma sex hormone and/or SHBG levels are related to fatness, and this is indeed the case. Many researchers have studied this association.

Menarche

Increases in circulating estrogens at puberty have been related to increased adiposity in females (Baumgartner & Roche, 1988), but it has long been debated which precedes the other; that is, do estrogens precede changes in adiposity, or do changes in adiposity precede (or trigger) increases in estrogens? In 1969 it was suggested that the onset of puberty depended on a critical level of body weight (Frisch & Revelle, 1969). Subsequently it was thought that a certain degree of fatness had to be achieved in order that menarche could occur; studies tended to focus upon fat as the determinant of menarcheal timing (Frisch & McArthur, 1974; Frisch, 1976). The relationship of the sex hormones to fatness was not considered. It was thought that fatness preceded the sex hormone surge which gave rise to menarche.

This "critical weight-fatness hypothesis" was in accordance with earlier evidence that fatter girls generally attain menarche at an earlier age than lean girls (Garn & Haskell, 1959), and these observations were valid (Garn, 1986). More recent investigations confirm the validity of these early observations (Frisancho & Flegel, 1982). However, it has since been found that there is no threshold level of fatness that can be termed "critical" for all girls with respect to menarche, nor is there any indication of a weight level that is "critical" (disallowing a period of acutely negative caloric balance resulting in amenorrhea) (Garn et al., 1983).

What appears "critical" is the duration and level of exposure to estrogens in women whose menarche occurs early. Two prospective studies of girls have shown significant inverse relationships between age at menarche and levels of estradiol, indicating that girls whose menarche occurred early produced greater amounts of estradiol than other girls (Vihko & Apter, 1984; Apter et al., 1984). Furthermore, one of these studies (Vihko & Apter, 1984), as well as two retrospective studies of adult females (MacMahon et al., 1982a; Smith L., 1989), indicate that women who experienced early menarche also produce greater quantities of estrone and estradiol as adults. In view of this, and insofar as recent studies suggest that it is actually maturational timing which has a greater immediate and long-term effect on levels of fatness than levels of fatness have on maturational timing (Garn, 1986; Garn et al., 1986a), it may very well be that it is the duration and level of exposure to estrogens during the peri-menarcheal period which determines both menarcheal timing and fatness. Specifically, it appears that the *integral* of estrogen production prior to menarche determines maturational timing and fatness, a hypothesis that only longitudinal observation can resolve.

Both ovarian and peripheral estrogen production would contribute to this integral during the peri-menarcheal period. The "critical fat hypothesis" is based fundamentally on the fact that adipose tissue provides a site for peripheral production of estrogens; that is, the less fat, the less estrogens. But given the relatively low (in relation to post-pubertal) levels of fatness in girls until the pre-menarcheal increase in *ovarian* estrogen production, peripheral estrogen production appears to be of little importance. Furthermore, the main estrogen produced via peripheral aromatization is estrone (from androstenedione) (Siiteri & MacDonald, 1973), and estrone is of limited biological activity in comparison to estradiol.

A recent route of study which may finally put to rest the "critical fat hypothesis" concerns influences which determine the timing of the onset of ovarian and peripheral estrogen production in the peri-menarcheal period. It appears that growth factors, such as IGF-1 (Insulin-Like Growth Factor-type 1), regulate ovarian steroidogenesis at puberty by mediating the effects of Growth Hormone (GH) (Reed & James, 1989). Growth Hormone

administration is known to be associated with reductions in WHR (Salomon et al., 1989), but pubertal decreases in WHR in girls are likely due more to the mediating effect of GH on ovarian steroidogenesis than a direct effect. Because GH deficiency is known to delay puberty (Sheikholislam & Stempfel, 1972), investigations of the influence of growth factors appear very promising in elucidating the variables determining menarche. Moreover, IGF-1 is known to act synergistically with FSH and LH in controlling the level of P-450 aromatase activity in human granulosa cells (Erickson et al., 1989) and, consequently, estradiol production (Erickson et al., 1990). It is estradiol which appears to be responsible for adipogenesis at puberty.

Adipogenesis

The effect of sex hormones on adipogenesis, the formation of new, differentiated adipocytes, is of considerable interest in view of changes in the amount and configuration of adipose tissue in girls at puberty. Adipose tissue mass is regulated not only by the amount of triglyceride in each adipocyte, but also by the number of adipocytes with the capacity to store triglyceride. Various studies *in vitro* have established the fact that mammals, including adult humans, possess adipocyte precursors capable of replication and complete differentiation into mature fat cells (Van et al., 1976; Van & Roncari, 1977; Roncari & Van, 1978a; Van & Roncari, 1978). This has also been confirmed *in vivo* (Klyde & Hirsch, 1979). The composition of the adipocyte precursor pool is thought to be an important determinant of the growth of adipose tissue, and appears to explain inter-regional and inter-individual differences (Dijan et al., 1983).

Adipocyte precursors are polyclonal mixtures. Preadipocytes obtained from the fat of experimental animals during tissue culture have been shown to demonstrate marked variation between colonies in the rate at which constituent cells accumulated lipid (Roncari et al., 1981; Dijan et al., 1983). An individual could therefore be predisposed to regional adiposity, or obesity in general, because he or she had in their precursor pool an unusual proportion of cells programmed for rapid differentiation (Wood, 1984). But while genetic susceptibility cannot be discounted in human variation of fat distribution, recent investigations have found an additive genetic effect of between 20-25% (Bouchard, 1988) and 24-31% (Selby et al., 1990) of remaining human variance in the amount of lower trunk fat and in the relative proportion of lower trunk versus extremity fat. Undoubtedly, other mechanisms are involved; the sex steroids may mediate genetic effects, as well as exert their own unique influence.

Studies *in vitro* have shown that 17ß-estradiol stimulates replication of human omental adipocyte precursors in culture (Roncari & Van, 1978b; Roncari, 1981), and a

similar investigation of rat adipocyte precursors found that administration of both 17ß-estradiol and progesterone stimulated differentiation and consequent new fat cell formation (Xu & Björntorp, 1987). Conversely, the male sex hormones testosterone and dihydrotestosterone do not influence growth or replication of human adipocyte precursors (Roncari, 1981) and actually appear inhibitory (Björntorp, 1987). Studies *in vivo* in the mouse have demonstrated that 17ß-estradiol promotes adipocyte hyperplasia (Bani & Bigazzi, 1984), while relaxin promotes adipocyte hypertrophy (Bianchi et al., 1986); the effect of 17ß-estradiol and relaxin together upon adipocyte precursors is differentiation in which both proliferation and lipid accumulation occurs (Bani-Sacchi et al., 1987). Other hormones thus might have synergistic effects upon adipocytes during growth and maturation. These studies suggest that in girls at puberty, the female sex steroids might be responsible for the regulation of adipose tissue storage capacity by increasing the number of adipocytes by inducing differentiation of adipocyte precursor cells.

The actual mechanism mediating sex steroid-enhanced preadipocyte multiplication at puberty in girls could be estrogen-induced production of paracrine/autocrine factors by adipose cells (Cooper & Roncari, 1989). Using preadipocytes from massively obese women, it has been shown that media grown in the presence of 17ß-estradiol contain significantly higher mitogenic activity, and that 17α-estradiol is not effective; these findings indicate that estrogens might exert their mitogenic effect on preadipocytes through local factors (Cooper & Roncari, 1989). Supporting this possibility are the observations that human preadipocytes release into culture mediums proteins mitogenic on other preadipocytes, and that preadipocytes from massively obese persons release higher mitogenic activity than cells from lean persons (Lau et al., 1987). Also, estrogens are known to bring about the release of mitogenic autocrine/paracrine polypeptides from the MCF-7 mammary carcinoma cell line (Aitken et al., 1985). In spite of the evidence still being circumstantial, estrogens are considered to be promoters of potential cancer cells (Bruning et al., 1988); this is not entirely surprising in view of their mechanism of action upon preadipocytes during puberty. It is worth noting that in the rat uterine endometrium, estradiol produces hyperplasia whereas anti-estrogens produce hypertrophy (Kang et al., 1975).

Evidence for the role of ovarian steroids in influencing regionally specific preadipocyte differentiation and conversion to adipocytes at puberty has been obtained utilizing a highly sensitive procedure to quantify differentiated and undifferentiated preadipocytes in normal and ovariectomized rats (Krakower et al., 1988). Results indicated that in normal female rats at puberty there was: (a) an increase in differentiated preadipocytes and in fat cell number; (b) enlargement of specific regional "female" depots,

including the femoral; and (c) a concomitant decline in the percentage of undifferentiated preadipocytes in all but the femoral depot. Ovariectomized animals were found to have: (a) reduced pubertal adipose growth in the femoral and parametrial depots; and (b) an unpreserved femoral undifferentiated preadipocyte pool. It was concluded that the association between ovarian hormones and body fat topography could be accounted for, in the female rat, by the finding that the femoral depot contains an ovarian-dependent infinite pool of fat cell precursors, and that sex steroids may determine the amount and recruitment rate of preadipocytes and, consequently, regional adipose tissue mass (Krakower et al., 1988). It is reasonable that similar events transpire in humans, and recent findings support this hypothesis.

It has been shown in a group of pre-menarcheal girls maturation-matched for Tanner's breast stage 2 that "free" levels of estradiol and testosterone (non-SHBG-bound) varied significantly with WHR (Ridder et al., 1990). Except for obese subjects, estrogen levels decreased with increasing WHR, and, in subjects with the greatest WHR, testosterone levels were highest. The authors concluded that decreasing WHR (a more gynoid form) is representative of pubertal endocrine activity in girls, and that this type of fat distribution is likely a result of ovarian activity (Ridder et al., 1990).

But one cannot claim that body fat topography is entirely ovarian hormone-dependent in girls. It is possible that development of regional fat depots is facilitated by peripheral estrogen production -- secondary to ovarian estrogen-dependent adipogenesis. Peripheral aromatization of androgens to estrogens could result in the newly formed estrogens acting upon neighboring cells by paracrine/autocrine factors, the resulting proteins increasing the number of preadipocytes by the same mechanisms. This may occur in adult obesity (Cooper & Roncari, 1989). That estrogen-induced production of paracrine/autocrine factors by adipocytes could account for regional variation in adiposity (via the production of mitogenic proteins) is supported by the fact that steroid metabolism is *not* uniform throughout the body; aromatase activity varies (Jasonni et al., 1981) and, correspondingly, so do the rates of conversion of androstenedione to estrone (Folkerd et al., 1982). Furthermore, adipose tissue represents a hormone pool in which steroid concentrations can be much greater than in serum (Feher & Bodrogi, 1982; Feher et al., 1982; Deslypere et al., 1985). But the strongest arguments are different peripheral aromatization rates in women with upper and lower body obesity (Kirschner et al., 1990) and greater serum levels of dehydroepiandrosterone sulfate (DHEA-S) in association with android fat distribution in adolescent girls (Katz et al., 1986; Hediger & Katz, 1986) and female primates (Kemnitz et al., 1989). Thus, if there is any credence at all to the "critical fat hypothesis" regarding menarche, it might be that a certain level of adiposity be reached

specifically in the gluteal-femoral areas, especially since this fat appears to be reproductively-related.

In summary, the onset of estrogen production during the peri-menarcheal period is related to an increase in overall adiposity. As a consequence, estradiol and fatness are significantly correlated in active, non-obese young women (Rice, 1988), just as in postmenopausal women (Jensen et al., 1985b; Barbosa et al., 1990). Furthermore, the gynoid distribution develops in girls at puberty; estrogen levels increase with decreasing WHR (in non-obese girls) (Ridder et al., 1990). These events appear to be due to an increase in the overall number of adipocytes, with specific regional variation in this response to estrogens. Both ovarian and peripheral mechanisms of estrogen production are implicated in adipogenesis.

Relative Androgenic/Estrogenic Balance

Much evidence supports the idea that body fat morphology is more a function of the androgenic/estrogenic balance than of estrogens alone. As discussed, androgen levels are elevated in adolescent girls of android body fat distribution (Hediger & Katz, 1986; Katz et al., 1986; Ridder et al., 1990). The onset of androgen secretion in the pubertal male or administration of exogenous testosterone to the hypogonadal male is accompanied by a decrease in percent body fat and a change in fat distribution towards the android type (Vague et al., 1974; Vague et al., 1978). Fat becomes localized at the abdomen, shoulders and neck. Testosterone is also known to reduce adiposity in animals (Robinson et al., 1987). Regionally-specific testosterone-dependent decreases in adipose tissue mass in humans appear to involve significant decreases in adipocyte number mainly in the trochanteric, but also deltoid, regions; these changes are supported by secondary decreases in adipocyte volume at these sites (Vague et al., 1978; Vague et al., 1984). It should be noted, however, that the methodology and assumptions required to determine changes in regional adipocyte number are difficult to validate. Regardless, it is clear that in both humans and animals, the degree of sexual maturation in males (dependent upon testosterone production) is inversely related to body fatness (Glass et al., 1987). High estradiol levels are positively related to overweight in adolescent boys for any given level of testosterone (Laskarzewski et al., 1983). Along with the previously-described effects of estrogens on adiposity in girls at puberty, observations such as these suggest that body fat topography is a function of relative androgenic/estrogenic balance, a hypothesis held by many (Evans et al., 1983; Hauner et al., 1987; Peiris et al., 1987a; Kissebah & Peiris, 1989). Specifically, it appears that in both men and women, adipose tissue distribution is android in the presence of an androgenic environment and/or when estrogenic influences

are weak. Conversely, fat distribution is gynoid in the presence of an estrogenic environment and/or when androgenic influences are weak.

Normal and atypical endogenous hormonal profiles support the hypothesis that body fat distribution is influenced by androgenic/estrogenic activity. In non-obese and obese adult women, android adiposity (high WHR and/or high WTR) is positively correlated with total serum testosterone (Hauner et al., 1987; Kirschner et al., 1990), free testosterone (Evans et al., 1983; Peiris et al., 1987a; Evans et al., 1988; Seidell et al., 1990), free testosterone to total testosterone ratio (Seidell et al., 1989a), the production rates of testosterone and dihydrotestosterone (Kirschner et al., 1990), and the production rate and metabolic clearance rate of androstenedione and the metabolic clearance rate of DHEA (Kurtz et al., 1987). Inverse associations of WHR with SHBG are numerous (Evans et al., 1983; Lapidus et al., 1986; Hauner et al., 1988; Haffner et al., 1989; Ridder et al., 1990; Kirschner et al., 1990). It is no surprise that in addition to a more masculine hormonal profile, android adiposity in women is also associated frequently with masculine characteristics of muscle tissue mass and morphology (Björntorp, 1988a; Björntorp, 1988b), as well as hirsutism and virilism (Mahesh & Greenblatt, 1983; Evans et al., 1988; Hauner et al., 1988). Clearly illustrating this are women with polycystic ovarian syndrome (PCOS), where hyperandrogenism is associated with android adiposity irrespective of obesity level (Evans et al., 1988), oligo-ovulation and/or hirsutism (Azziz, 1989). Adult men are predominantly android, but of the few who are of gynoid fat distribution, plasma estradiol is elevated (Sparrow et al., 1980).

The effects of exogenous hormonal intervention also support the preceding observations. Shifts toward gynoid fat topography can be experimentally induced in males by treatment with estrogens (Lafontan et al., 1985). Male-to-female transsexuals given estrogens display female SHBG levels (Damewood et al., 1989) and gynoid characteristics (Vague et al., 1984). Conversely, female-to-male transsexuals given androgens display android characteristics (Vague et al., 1984). Indeed, one of the many goals of endocrine therapy for sex-changes is to provide feminization or masculinization of subcutaneous adipose tissue, and this is most often achieved (Prior et al., 1989).

The theoretical concept of an androgen/estrogen balance thus has empirical support. Because a ratio is sensitive to changes to either numerator or denominator, total amounts of androgens and estrogens may not be as important as their relative activity. Regional adiposity which is clearly opposite to that which would be expected based upon outward expression of gender is probably most often not associated with absolute dominance of, for example, androgens over estrogens, but rather, *relative* dominance of one over the other.

Perhaps the best way to illustrate the effect of this is by way of changes which occur at menopause.

Menopause results in a shift toward a more masculine hormonal profile, in keeping with the observed concomitant shift from gynoid to android adiposity. It is important to realize that adipocytes, like any steroid-sensitive cells, are dependent upon a continuing supply of steroid hormone in order to function in a certain role or at a certain level (Tepperman & Tepperman, 1987). Gynoid adiposity appears to be dependent upon female sex steroids; with the cessation of ovarian hormone production and consequent alteration of androgen/estrogen balance at menopause, it is no surprise that fat distribution changes. Contributing to this convergent morphology of the sexes in middle age is the age-associated decline of testosterone and concomitant increase of SHBG in men (Vermeulen & Verdonck, 1972; Duerr & Pirke, 1973), which indicates increased estrogenic relative to androgenic activity.

It is highly relevant that postmenopausal women undergoing estrogen replacement therapy decrease in fat mass (Jensen et al., 1986) and that specifically it is android fat which decreases (Seidell et al., 1989b). Furthermore, postmenopausal women reporting replacement estrogen use at any point during their menopausal years have a lower WHR than women reporting "never-" use of replacement estrogens (Kaye et al., 1990). These observations suggest that estrogens counteract the menopause-associated change to android adiposity by shifting androgen/estrogen balance back towards premenopausal levels.

Obesity

Obesity confounds the relationship of sex steroids with regional adiposity because, in general, obesity is characterized by abnormal sex steroid levels, and these differ between men and women. Obesity is also usually associated with increased abdominal adiposity; the abdominal area is the most responsive to changes in weight (Garn et al., 1987), which might explain why obesity correlates with WHR in men and women.

Both plasma levels and metabolism of estrogens are elevated in obesity. These observations are explained in part by close relationships between excessive body weight and metabolic transformation of androstenedione to estrone (MacDonald et al., 1978). Normal in postmenopausal women, this pathway of metabolism becomes even more active in the obese due to the increased fat mass available for aromatization (Hausknecht & Gusberg, 1973; Siiteri & MacDonald, 1973; Longcope, 1974; Riskallah et al., 1975; MacDonald et al., 1978). Thus, estrone levels and respective aromatization rates of androstenedione and testosterone to estrone and estradiol correlate with adiposity in postmenopausal women (Vermeulen & Verdonck, 1978; Vermeulen & Verdonck, 1979;

Jensen et al., 1985b; Longcope et al., 1986). In premenopausal obese women, estradiol levels are markedly elevated, and the estrone production rate increases with body weight; peripheral aromatization of androstenedione to estrone increases as a function of obesity (Kirschner et al., 1981). Obese men are characterized by elevated plasma estradiol and estrone levels (Schneider et al., 1979; Kley et al., 1979; Brind et al., 1990), increased estradiol and estrone production, and increased rates of aromatization of androstenedione to estrone and testosterone to estradiol (Schneider et al., 1979).

Why then, do obese men and women tend to display greater degrees of android adiposity? This relationship is not consistent with the association of estrogens with gynoid adiposity. The answer appears to be related to the well-recognized inverse relationship of obesity with SHBG (Peiris et al., 1989); obesity results in a decrease of SHBG in both men (Stefanick et al., 1987) and women (Davidson et al., 1981). It is not known, however, why SHBG decreases in obesity.

In women, as a consequence of subnormal levels of SHBG, levels of both free estradiol and free testosterone are significantly elevated (Zumoff, 1988), perhaps explaining why both overall and android adiposity tend to be increased in obesity. Increased tissue exposure to unbound androgens has already been noted as related to android fat distribution, and it has long been known that obese women have increased androgen metabolism (Bassoe et al., 1969). Furthermore, there is circumstantial evidence indicating preferential adipose tissue uptake of androgens: android obese women have significantly depressed levels of urinary DHEA (Sonka et al., 1965), and the abdominal fat of obese women contains a large pool of tissue-bound DHEA (Feher & Halmy, 1975a; Feher & Halmy, 1975b). It has been speculated that low urinary DHEA excretion in android obese females reflects greater binding to DHEA to abdominal adipose tissue (Hediger & Katz, 1986).

But the argument of low SHBG resulting in increased levels of unbound androgens does not hold for obese men: decreased SHBG is associated not only with elevated estrogens (as noted above) but also with *decreased* plasma testosterone levels (Glass et al., 1977; Amatruda et al., 1978; Kley et al., 1979; Schneider et al., 1979) and subnormal levels of free testosterone (Amatruda et al., 1978; Schneider et al., 1979; Barbato & Landau, 1984; Zumoff, 1988). That androgens decrease and estrogens increase is completely contrary to what would be expected with a decline in SHBG, and begs explanation. It has been suggested that these findings in obese men represent a state of hypogonadotropic hypogonadism (HHG) induced by increased aromatization due to additional adipose tissue; that is, a state of feedback suppression (Zumoff, 1988). This

hypothesis, however, still does not explain why obese men are generally characterized by upper body adiposity, an androgenic trait.

While the association of female obesity with a hyperandrogenic/hyperestrogenic state is generally recognized (Azziz, 1989), is it reasonable not to qualify fat distribution when studying or discussing the hormonal milieu of obesity? A recent investigation has indicated specifically that women with android obesity, in comparison to women of gynoid obesity, have higher androgen production rates and higher circulating levels of both free testosterone and free estradiol (Kirschner et al., 1990). Women with gynoid obesity were found to have greater rates of peripheral conversion of androstenedione to estrone and greater urinary excretion rates of estrone (Kirschner et al., 1990). Other studies have shown that android obesity, again specifically in comparison to gynoid obesity, is characterized by elevated secretion of testosterone, androstenedione and cortisol after stimulation by adrenocorticotropin (ACTH) (Björntorp, 1988b). Also, women with android obesity have elevated urinary secretion of 17-hydroxy- as well as 17-ketosteroids (Krotkiewski et al., 1966). There are thus significant differences in androgen and estrogen levels, metabolism and excretion rates between android and gynoid obesities, clear evidence that obesity is not a homogeneous entity.

It is interesting to note that android obese women are frequently oligo-ovulatory or amenorrheic (Azziz, 1989), while gynoid obese women are not. This difference might be explained by the hypothesis that there is a critical level or production rate of estrone -- dependent on adequate adiposity -- for the normal mature function of the hypothalamic-pituitary-ovarian axis (Loughin et al., 1985). Such a hypothesis is suggested by the finding of suppressed estrone levels and decreased conversion of androstenedione to estrone in amenorrheic women undergoing reductions in adiposity (Loughin et al., 1985). Insofar as the above evidence (Kirschner et al., 1990) indicates that android obese women are deficient in estrone production (relative to gynoid obese women), such a theory is entirely plausible. Moreover, this theory has important implications for the development of gynoid adiposity in peri-menarcheal girls, something speculated on already, in that gynoid fat-dependent estrone production could be a requirement for menarche.

Regional Characteristics of Adipocytes

The link between body fat distribution and the steroid hormones must involve inter-regional differences in the cellular characteristics of adipocytes (Table 2-2). In men, android body fat is associated with larger size of abdominal adipocytes (Ashwell et al.,

TABLE 2-2. Regional Characteristics of Adipocytes in Men and Pre- and Postmenopausal Women.[1]

Adipocyte Region	Cellular Characteristics	Premenopause (Gynoid)	Postmenopause (Android)	Men (Android)
Abdominal and Intra-abdominal	normal	***		
	hypertrophy		***	***
Gluteal-Femoral	normal			***
	hypertrophy	***	***	
	hyperplasia	***	***	

[1]comparisons relative to other regions *within* groups

1978; Kissebah et al., 1982), and a greater percentage of this is located internally (Grauer et al., 1984; Ashwell et al., 1985). Men also have larger omental and mesenteric fat cells than premenopausal women, in whom omental fat cell size has been found to be the smallest (Fried & Kral, 1987). Premenopausal women are generally of gynoid distribution and consistently display greater size of gluteal and femoral adipocytes (Rebuffé-Scrive & Björntorp, 1985; Fried & Kral, 1987); there is also a greater number of fat cells in these regions (Lanska et al., 1985a; Vansant et al., 1988). Notably, in premenopausal women, both SHBG (Evans et al., 1983) and androgen levels (Grenman et al., 1986) correlate inversely with abdominal fat cell volume. These findings indicate that large abdominal adipocyte size and android adiposity are not "feminine" or estrogen-dependent traits. Gluteal-femoral fat cell hypertrophy, such that these fat cells are larger than those in the abdominal region, is a trait which characterizes premenopausal women; this difference has been found to disappear with menopause due to an increased size of abdominal adipocytes which occurs with menopause (Rebuffé-Scrive & Björntorp, 1985). Like men, women of android distribution have significantly larger abdominal adipocytes than women of gynoid distribution (Evans et al., 1983; Hartz et al., 1984; Vansant et al., 1988), but in both men and women, subcutaneous adipocytes are larger than intra-abdominal adipocytes (Rebuffé-Scrive et al., 1989). Men and women of android body fat distribution display abdominal adipocyte hypertrophy (Lanska et al., 1985a). There are thus two fat depots of special importance, one located in the abdominal region and the other located in the gluteal-femoral region, and two mechanisms which can influence adipose tissue mass, adipocyte hyperplasia and adipocyte hypertrophy.

Regional Differences in Adipocyte Metabolism

Not only do steroid hormones affect differentiation and growth, they also affect the adaptation of cells to new metabolic demands. Thus, the influence of steroid hormones on adipocyte metabolism is of considerable interest in view of their inevitable effect upon adipocyte size. The following will illustrate how steroid hormones are involved in the maintenance of regional adipose tissue depots in adulthood through effects on fat cell size. It is suggested that inter-regional differences in the *number* of adipocytes reflect, in general, steroid hormone-mediated events which transpire in adolescence and early adulthood. This, however, does not rule out the possibility of steroid hormone-mediated adipogenesis in adulthood, in which both estrogen (Roncari, 1981) and progesterone (Xu & Björntorp, 1987) have been implicated.

Adipocyte size is a function of the metabolism of fat cells, and the main function of adipose tissue is to store triglyceride during periods of affluence and to release the stored lipids as fatty acids when needed. Uptake of triglyceride (triacylglycerol) into adipose tissue (lipogenesis) is controlled by the rate-limiting enzyme lipoprotein lipase (LPL); it hydrolyzes the triacylglycerol transported in very low density lipoproteins (VLDLs) and chylomicrons, thereby making their triacylglycerol fatty acids available for uptake in various tissues, including adipose tissue. Specifically, LPL hydrolyzes triacylglycerol to diacylglycerol to monoacylglycerol and then into free fatty acids and glycerol. Uptake into adipose tissue is accomplished by esterification (fatty acid biosynthesis). The release and efflux of fatty acids from adipose tissue (lipolysis) is accomplished by action of hormone-sensitive lipase; this is the key enzyme responsible for hydrolysis of triacylglycerol in adipose tissue to free fatty acids and glycerol. Both lipogenesis and lipolysis display sex and regional differences; both aspects of adipocyte metabolism have been studied. The following subsections will deal with both lipolysis and lipogenesis individually, and the direct effect of estrogens on these processes will be discussed in absolute terms, mainly in view of experimental evidence concerning tissue cultures and animals.

Lipolysis.

Investigations of regional adipocyte metabolism in humans have shown that the larger size of abdominal adipocytes is associated with a greater rate of lipolysis *in vitro* (Arner et al., 1981; Kissebah et al., 1982; Smith U. et al., 1979). Abdominal fat cells also show a diminished sensitivity to the anti-lipolytic action of insulin (Bolinder et al., 1983). Omental adipocytes have been found to be more sensitive to lipolytic agents than subcutaneous fat cells, and are associated with lower activities of LPL than the latter (Lithell & Boberg, 1978; Smith U., 1985). Such studies help to explain the high frequency of impaired glucose tolerance found in android obesity; it may be that an

increased or greater rate of free fatty acid release *in vivo* could inhibit glucose uptake by peripheral tissues, thus contributing to insulin resistance (Evans et al., 1988).

Several studies have looked directly at the effects of catecholamines on regional adipocyte lipolysis in humans. Adipocytes in the abdominal subcutaneous regions have been found to be more responsive than those of femoral regions to norepinephrine, and norepinephrine has been found to be more lipolytic than epinephrine (Lafontan et al., 1985). The femoral subcutaneous regions are particularly insensitive to epinephrine-stimulated lipolysis *in vivo* (Lafontan et al., 1979), and differences in epinephrine-stimulated adenylate cyclase activities in adipocytes withdrawn from various regions have been demonstrated *in vitro* (Kather et al., 1977). Furthermore, there appear to be both sex and regional differences in the distribution of activity of adipocyte adenylate cyclase (Kather et al., 1977) and, since human adipocytes possess both ß- and α_2-adrenoreceptors coupled respectively in a positive and negative fashion to plasma membrane adenylate cyclase, adenylate cyclase activity is of prime importance in controlling the lipolytic activity of adipocytes (via cyclic-AMP production) through protein-kinase and hormone-sensitive lipase activation (Lafontan et al., 1985).

That testosterone may enhance abdominal α_2-adrenoreceptor activity is suggested by the finding that free testosterone is significantly correlated with abdominal adipocyte volume (Evans et al., 1983). This has recently been confirmed in castrated male hamsters: testosterone treatment *in vivo* promotes α_2-adrenoreceptor-mediated antilipolysis to a greater extent than it increases the ß-adrenergic lipolytic effect of catecholamines (Perquery et al., 1988), which could explain why men (and androgenic women) accumulate adipose tissue in the abdominal region. However, an *in vitro* study of adipose precursor cells from male rats suggests that testosterone stimulates lipolysis at the ß-adrenoreceptor level and that both testosterone and dihydrotestosterone increase adenylate cyclase activity (Xu et al., 1990). Although the authors of this latter study state that the α_2-adrenergic action of testosterone is "weak, perhaps nonexistent", results of the former *in vivo* study contradict this position. In middle-aged men, testosterone has been found to stimulate norepinephrine-induced abdominal lipolysis (Rebuffé-Scrive et al., 1989b), but the specific effect of testosterone on α_2/ß-adrenergic control of lipolysis remains to be clarified.

Direct human evidence regarding the effects of estrogens on adipocyte lipolysis is also lacking. In rats, estrogens increase *in vitro* catecholamine-stimulated lipolysis in adipocytes by enhancing hormone-sensitive lipase activity (Wade et al., 1985). The strong regulatory effects of estradiol on enzyme activities in rat adipose tissue are mediated in turn by cyclic-AMP (Tomita et al., 1984), as in humans, which is thought to be the likely mechanism whereby the estrogens promote lipolysis in rat fat cells (Pasquier et al., 1988).

Treatment of rats with 17ß-estradiol alone or in combination with progesterone has been shown to facilitate lipolysis with regional variation in response; progesterone alone appears to have no effect (Rebuffé-Scrive, 1987). It has been speculated that both estradiol and testosterone are lipolytic in the abdominal region (Rebuffé-Scrive, 1988) but, as noted above, the specific effect of testosterone remains to be elucidated.

Lipogenesis.

It appears that the sex hormones also account for sex and regional differences in LPL regulation, and therefore lipogenesis. In rats, regional differences have been demonstrated; LPL activity is highest in gonadal regions and lowest in subcutaneous sites (Cryer & Jones, 1980; Gruen & Greenwood, 1981). There is also regional variation of LPL activity in humans. The gluteal-femoral region in non-obese premenopausal women with normal ovarian and adrenal functions is characterized by elevated LPL activity (relative to the abdominal, and other, regions) (Rebuffé-Scrive et al., 1985a). Moderately obese women have higher activities of LPL in the gluteal and femoral regions than in the abdominal or triceps region in parallel to the cell size differences (Bosello et al., 1984; Lithell & Boberg, 1978), and a study of morbidly obese men and women also found that fat cell size and LPL activity were higher in the femoral region than in abdominal and gluteal subcutaneous deposits in women, with men showing less marked regional variations (Fried & Kral, 1987). Other studies have reported higher adipose tissue LPL activity in the gluteal region of females compared to males (Björntorp et al., 1975; Taskinen & Nikkilä, 1981; Taskinen et al., 1980); however, none of these latter investigations considered regional differences in LPL activity between the sexes. Men, in general, demonstrate low femoral adipocyte LPL activity; this is even lower than that in postmenopausal women (Rebuffé-Scrive et al., 1987). Abdominal adipose tissue LPL activity is higher than that of the femoral region in men (Rebuffé-Scrive et al., 1987).

Available data regarding direct sex steroid effects on adipose tissue and plasma LPL activity in humans are contradictory. However, endogenous sex steroid levels have only been measured in a few investigations; most studies have utilized exogenous sex steroids and have looked specifically at their effect on postheparin plasma LPL. A recent investigation of *endogenous* sex steroid levels and their relationship with LPL activity in obese pre- and postmenopausal women found estradiol to be a major negative regulator of fasting adipose tissue LPL, independent of degree of obesity (Iverius & Brunzell, 1988). Supporting this work is animal research. Estradiol has been found to reduce LPL activity in both intact and gonadectomized animals (Dark et al., 1984; Gray & Wade, 1980; Hamosh & Hamosh, 1975; Steingrimsdottir et al., 1980; Wade et al., 1985; Wilson et al., 1976) as well as influencing hormone-sensitive lipase (Krakower et al., 1988) while

progesterone, only affecting female adipose tissue (Gray & Wade, 1980), counteracts the negative estrogen effects on LPL (Gray & Wade, 1980; Steingrimsdottir et al., 1980). Androgens also appear to inhibit LPL activity, as administration of testosterone shows (Rebuffé-Scrive et al., 1989b), but it has been suggested that under *in vivo* conditions androgen inhibition of adipose tissue LPL results from estradiol formed by aromatization rather than from a direct effect (Gray et al., 1979).

That progesterone counteracts estrogen effects on LPL might be due to competition by progesterone for glucocorticoid receptors; progesterone effectively competes with glucocorticoids present in human adipose tissue (Rebuffé-Scrive et al., 1985b). Interestingly, progesterone alone has no effect on LPL induction in *in vitro* experiments, but addition of cortisol to the culture medium (containing progesterone) increases the cortisol effect on LPL activity (Cigolini & Smith, 1979). However, an independent and directly anabolic role for progesterone in adipose tissue lipogenesis has been suggested on the basis of rat experiments (Mendes et al., 1985). Progesterone clearly reverses the adiposity-reducing actions of estradiol in rats, but in other animals (hamsters) the action of both estradiol and progesterone together is weight loss (Bhatia & Wade, 1989). Such synergistic effects cannot be discounted in humans, but it is becoming apparent that the respective positive and negative effects of progesterone and estrogen are regionally specific.

In premenopausal women, progesterone appears to increase femoral LPL activity under *in vivo* conditions (Rebuffé-Scrive et al., 1983), and this is consistent with the large femoral adipocytes in these women. Such an effect of progesterone has also been observed in rats (Steingrimsdottir et al., 1980). Thus, a lack of progesterone in postmenopausal women and greater levels of testosterone in men might explain the smaller size of femoral adipocytes in these groups (Rebuffé-Scrive, 1988). This also has implications for fat distribution in oligo-ovulation and amenorrhea: decreased or low progesterone production could result in less fat being accumulated in the typical gluteal-femoral depots and more fat being accumulated abdominally, especially since the lipolytic effect of estrogen on abdominal adipocytes would be stymied due to low estrogen levels. Importantly, when progesterone (and estrogen) levels are low, such as in women with PCOS, there are no differences in LPL activity between abdominal and femoral fat depots (Rebuffé-Scrive et al., 1989a).

It should be noted that estrogen appears to be required for the progesterone effect on femoral LPL activity; the formation of specific progesterone receptors for translocation of the hormone to the nucleus requires estrogen (Wade, 1976; Gray & Wade, 1979). Furthermore, in the ovariectomized rat uterus, estradiol and non-steroidal anti-estrogens

(tamoxifen and monohydroxytamoxifen) increase progesterone receptor content (Jordan & Prestwich, 1978). Of even more interest is the fact that progesterone is implicated in the control of peripheral aromatase activity (Newton et al., 1986); progesterone could hypothetically promote production of the estrogens required for formation of its own receptors.

Reproductive Status and Metabolism

The role of the sex steroids in mediating the interplay between lipolysis and lipogenesis is particularly implicated by alterations of reproductive status, something which several investigations of regional adipose tissue metabolism in women have considered. In a study of both pregnant and nonpregnant women, lipid assimilation was shown to be favoured in the femoral depot over the abdominal depot due to elevated LPL activity in the femoral depot, and lipolysis was significantly less in the femoral region (Rebuffé-Scrive et al., 1985a). These results were observed in both the pregnant and nonpregnant women, and are in keeping with previous results reported for premenopausal women. However, during lactation and late pregnancy, basal lipolysis increased significantly in the femoral region, and LPL activity decreased significantly (relative to the nonpregnant women) (Rebuffé-Scrive et al., 1985a; Smith U., 1985). In the pregnant women, there was, in fact, no difference between the lipolytic rate of the abdominal and femoral regions during lactation, and the decreased femoral LPL activity acted synergistically to increase lipid mobilization from this depot considerably during lactation (Rebuffé-Scrive et al., 1985a). Thus, the previous pattern favoring fat accumulation in the femoral adipocytes in nonpregnant and pregnant women apparently changes in *late* pregnancy and during lactation so that this depot can be effectively utilized. This study indicates that femoral adipose tissue may serve as an important source of energy supply during lactation. Such a possibility is supported by the characteristic preponderance of the femoral fat depots in women and by the contrasting fact that men usually only have a small femoral fat depot (Krotkiewski et al., 1983; Sjöström et al., 1972).

But the foregoing does not openly indicate the role of the sex steroids. The characteristic metabolic features of the femoral region could be due to inherent characteristics of adipocytes in this region and/or specific effects of female sex hormones. A study of femoral, mammary and abdominal adipocyte metabolism in pre- and postmenopausal women provides a clearer look at the role of the female sex steroids. In premenopausal women femoral adipocytes were found to display greater LPL activity than abdominal or mammary adipocytes, while lipolytic responsiveness and sensitivity of the latter two was greater than in femoral tissue (Rebuffé-Scrive et al., 1986). In

postmenopausal women no differences were found among the three regions; consequently, menopause seems to be associated with a decrease of not only the elevated LPL activity of femoral adipocytes, but also the high lipolytic response in abdominal and mammary adipose tissue (Rebuffé-Scrive et al., 1986). Of further interest is the finding that intra-abdominal fat depots, in comparison to subcutaneous abdominal tissue, are similar in both men and postmenopausal women (Rebuffé-Scrive et al., 1989). Thus, the female sex steroids appear to elevate LPL activity in femoral adipocytes, causing them to become enlarged, and stimulate lipolysis in abdominal and mammary adipocytes. These results, summarized in Table 2-3, support the hypothesis that the secondary sex characteristics of female adipose tissue distribution might be caused by regionally specific effects of sex steroids on adipocyte metabolism.

TABLE 2-3. Adipocyte Metabolism and Reproductive Status.

Region	Pre-menopause	Pregnancy	Lactation	Post-menopause	Men
Relative Lipolytic Enzyme Activity According to Adipocyte Region					
Abdominal	moderate	moderate	moderate/high	high	high
Gluteal-femoral	low	low/moderate	moderate/high	high	moderate
Relative Lipoprotein Lipase (LPL) Activity According to Adipocyte Region					
Abdominal	low	low	low	moderate	moderate
Gluteal-femoral	high	high	low	low	low

Evidence of the direct role of the sex steroids in regional fat cell metabolism is provided by a study of middle-aged men and postmenopausal women who underwent sex hormone treatment. Before hormone treatment both fat cell size and LPL activity were found to be similar in subcutaneous abdominal and femoral regions in postmenopausal women (Rebuffé-Scrive et al., 1987), and this clearly differs from the previously noted characteristics of young women. The difference appears to be due to hormonal status, because estrogen and progestin administration to the postmenopausal women significantly enhanced femoral, but not abdominal, adipocyte LPL activity (Rebuffé-Scrive et al., 1987). The role of female sex hormones in lipid accumulation and enlargement of the femoral depot is further illustrated by the observation that estrogen administration to males with prostate carcinoma leads to an enlargement of the gluteal-femoral cells (Krotkiewski & Björntorp, 1978). Treatment of the middle-aged men with testosterone increased basal lipolysis in the abdominal cells, supporting the regulatory role of male sex steroids in

abdominal adipocyte lipolysis; no effect on lipolysis in postmenopausal women was noted with female sex hormone treatment (Rebuffé-Scrive et al., 1987).

Summary

The distribution of adipose tissue clearly varies with degree of maturation and by gender. Body fat distribution overlaps throughout childhood, but major changes occur at puberty: sexual dimorphism in terms of both the amount and distribution of body fat becomes apparent. Males decrease in fatness and take on an android (abdominal) distribution, females display essentially no change in fatness but take on a gynoid (gluteal-femoral) distribution. While the android pattern of adipose tissue distribution dominates in males throughout adulthood, the gynoid pattern of women changes at middle age. Menopause results in a shift from gynoid to android body fat topography, and fat distribution thus overlaps again later in life. These observable shifts of distribution at puberty and menopause strongly imply involvement of the sex steroids in mediating these effects.

Physiological changes in both the levels and rates of synthesis of steroid hormones at puberty and menopause occur concomitant to shifts of body fat topography. Moreover, sex steroids have been shown to be directly involved in mediating effects upon regional adipose tissue depots at these times. In pubertal males, androgens appear to promote an android distribution through effects on both the number and size of adipocytes. Testosterone seems to decrease adipocyte number in all but the abdominal area, resulting in a relative abdominal "hypercellularity". In pubertal females, the gynoid shape appears dependent upon estrogen and progesterone which seem to increase the size and number of gluteal-femoral adipocytes relative to abdominal adipocytes.

In adulthood, body fat distribution appears influenced by androgenic/estrogenic balance. Hormonal manipulation supports this contention. Increased androgenic/estrogenic activity is associated with android body fat topography, while decreased androgenic/estrogenic activity is associated with gynoid body fat topography. These observations may be noted in either sex. Regional differences in the metabolic and cellular characteristics of adipocytes exist, and these are particularly apparent at the abdominal and gluteal-femoral areas. The android "male pattern" distribution is associated with large abdominal adipocytes relative to other areas, while the gynoid "female pattern" distribution is associated with a greater number and larger size of gluteal and femoral adipocytes relative to other areas.

The female sex steroids have metabolic effects which serve to maintain the gluteal-femoral distribution of adipose tissue in women when these hormones are dominant.

Specifically, they seem to elevate LPL activity in the gluteal-femoral areas as well as elevate lipolysis in the abdominal areas. Progesterone is implicated in the former and estrogen in the latter. That the gluteal-femoral area might play a specific role in reproductive function is suggested by the fact that during late pregnancy and lactation, lipolysis increases and LPL activity decreases; this depot becomes mobilized. Such a metabolic shift also occurs at menopause, but hormone replacement therapy counteracts this phenomenon. Reproductive viability appears necessary for protection of the gluteal-femoral depot.

Section II: Smoking, Sex Hormones and Regional Adiposity

Relationship of Smoking to Sex Hormone Balance

Women

Menopause is a naturally occurring phenomenon which induces a shift in body fat topography toward the android type by altering relative androgenic/estrogenic balance; the previous section has described in detail how this might occur. Based upon this, it may be hypothesized that an artificially-induced increase in relative endogenous androgenic/estrogenic activity could also induce a shift towards android body fat distribution, with the implication of increased health risk. One manner in which this may occur is by smoking; there is extensive epidemiological evidence that women who smoke cigarettes are relatively estrogen deficient.

It has been shown frequently that smoking is associated with early natural menopause and menopausal symptoms (Jick et al., 1977; Kaufman et al., 1980; Lindquist & Bengsston, 1979; Baron, 1984; Hartz et al., 1987; Willet et al., 1983). A strong dose-response relationship of smoking with hirsutism in women has been demonstrated, as well as a significantly greater risk of oligomenorrhea (Hartz et al., 1987). With respect to fertility: (a) there has been found a direct relationship between cigarette usage per day and time required to become pregnant (Garn, 1985b); (b) smoking is associated with the inability to become pregnant (Tokuhata, 1968); and (c) pregnant women who smoke have lower serum estrogens than nonsmokers (Mochizuki et al., 1984; Bernstein et al., 1989). A study of the association between mothers' smoking habits and the frequency of dizygotic twinning also provides evidence for estrogen deficiency in women who smoke (Olsen et al., 1988).

Decreased estrogen production in premenopausal female smokers has been proposed based on the finding of reduced luteal phase, but not follicular phase, urinary excretion of estrone, estradiol and estriol (MacMahon et al., 1982b). However, doubts

have been raised about the actual difference in total estrogen production because 2-hydroxyestrone, a major urinary excretory estrogen, was not measured (Zumoff, 1983). Increased 2-hydroxylation of estradiol has been reported in female smokers (Michnovicz et al., 1986), which indicates increased degradation of estrogens. A recent investigation of urinary estrogens has demonstrated greater 2-hydroxyestrone excretion and concomitantly lower estriol excretion in female smokers, but no difference in total urinary measurements of estrone, estradiol, estriol, 16α-hydroxyestrone and 2-hydroxyestrone between smokers and nonsmokers (Michnovicz et al., 1988). It should be recalled that there are two metabolic (and irreversible) pathways for estrogens -- 2-hydroxylation and the 16α pathway. The 16α pathway product estriol retains estrogen agonist activity while the 2-hydroxylated catechol estrogen 2-hydroxyestrone possesses minimal peripheral estrogenic activity. Thus, the latter investigation (Michnovicz et al., 1988) strongly indicates increased metabolism of estrogens as a mechanism of deactivation versus decreased estrogen production. All the above refer to *in vivo* investigations.

It has been put forth that the endocrine consequences of smoking include an "anti-estrogenic" effect (Baron, 1984). While this suggestion has evolved principally on the basis of data on premenopausal women (as noted above), observations of postmenopausal women also support this concept. There is some evidence indicating an anti-estrogenic effect of smoking in postmenopausal women (Baron et al., 1988) and studies of postmenopausal smokers and nonsmokers during hormone-replacement therapy have demonstrated significantly greater hepatic metabolism of estrogens among smokers (Jensen et al., 1985a; Jensen & Christiansen, 1988). Comparisons of endogenous levels of sex hormones between smokers and nonsmokers have consistently shown levels of the adrenal androgen androstenedione to be significantly elevated in smokers, with no significant difference in estradiol and estrone between groups (Friedman et al., 1987; Khaw et al., 1988; Cauley et al., 1989; Longcope & Johnston, 1988). Comparisons are inconsistent for DHEA-S and testosterone; DHEA-S and testosterone were elevated in the smoking groups of two studies (Khaw et al., 1988; Friedman et al., 1987), but there was no significant difference in levels of these androgens between groups in two other studies (Longcope & Johnston, 1988; Cauley et al., 1989). In any case, there is an increase in relative androgenic/estrogenic activity in women, be it by increased androgens or decreased estrogens.

Epidemiologic studies of osteoporosis in postmenopausal women also provide some evidence for an anti-estrogenic effect of smoking. Postmenopausal smokers are at greater risk for osteoporosis than nonsmokers (Daniell, 1976; Williams et al., 1982; Paganini-Hill et al., 1981; Hussey, 1976), and a hypoestrogenic state is a well-accepted

risk factor for osteoporosis (Friedman et al., 1987), since serum estradiol and serum estrone are positively correlated to bone mineral density (Murakami et al., 1979; Cauley et al., 1986). It should be noted, however, that other nonestrogenic mechanisms have been proposed to explain the effect of smoking on bones (Baron, 1984; Friedman et al., 1987).

Final evidence for an anti-estrogenic effect of smoking in women (both pre- and postmenopausal) concerns decreased incidence rates of estrogen-dependent cancers in female smokers (Editorial, 1986). Endometrial cancer occurs less frequently in women who smoke than in nonsmokers (Lesko et al., 1985; Lawrence et al., 1987; Weiss et al., 1980; Franks et al., 1987); this phenomenon has also been noted in women on noncontraceptive estrogen replacement therapy (Franks et al., 1987). Breast and colorectal cancer incidence rates are also decreased in female smokers (Vessey et al., 1983; Sandler et al., 1988). Such evidence strongly supports direct evidence of an anti-estrogenic effect.

Antiestrogenic Effect Mechanisms

The mechanisms by which smoking may act to alter estrogen levels in women are not fully understood. An anti-estrogenic impact of smoking could in principle occur at any point of estrogen metabolism from secretion to the final organ result. Lower estrogen levels after one year of exogenous estrogen therapy in postmenopausal smokers (Jensen et al., 1985a; Jensen & Christiansen, 1988) and greater 2-hydroxylation of estrone (from estradiol) in premenopausal smokers (yielding the relatively inactive catechol estrogens 2-hydroxyestrone and 2-methoxyestrogen) (Michnovicz et al., 1988; Michnovicz et al., 1986) suggest that degradation of estradiol is increased in smokers. Furthermore, smoking has been found to influence thyroid function -- albeit inconsistently -- and changes in thyroid function may alter androgenic and estrogenic steroid metabolism (Gofin et al., 1982; Sepkovic et al., 1984). The possibility of a peripheral (extraovarian) biologic mechanism such as induction of microsomal oxidative systems induced by smoking has been suggested as contributing towards increased estrogen degradation (Baron, 1984; Franks et al., 1987); steroid hormones are metabolized in microsomal oxidative systems at least parts of which are induced by smoking (Jusko, 1978; Conney, 1967; Conney, 1971). Other postulated extraovarian mechanisms for an anti-estrogenic effect of smoking include alteration in the binding of estrogen receptors, and inhibition of peripheral conversion of androgens to estrogens (Lawrence et al., 1987).

There is evidence supporting the suggestion that smoking inhibits the peripheral conversion of androgens to estrogens. This evidence also indicates that ovarian estrogen synthesis may be inhibited by smoking. Evaluation of the effects of aqueous extracts of cigarette smoke on cultures of human granulosa cells from normal ovulatory women

indicated that cigarette smoke inhibited the conversion of androstenedione to estradiol in a dose-dependent manner (Barbieri et al., 1986b) and constituents of tobacco (nicotine, cotinine and anabasine) have also been shown to inhibit conversion by aromatase of androstenedione and testosterone to estrogens *in vitro* (Barbieri et al., 1986a). In each case, removal of the aqueous cigarette extracts or tobacco constituents from their respective culture mediums demonstrated complete reversal of aromatase inhibition. Thus, at least in the case of peripheral aromatization, smoking inhibition of androgen conversion may only be operative while the inducing exposure is present (Baron, 1984; Franks et al., 1987), and the anti-estrogenic effect of smoking reversible after cessation of smoking. This may not be the case with smoking inhibition of *ovarian* granulosa cell aromatization because human and animal studies indicate ovarian damage and/or atrophy as a result of smoke-exposure (Mattison & Thorgeirsson, 1978; Baron, 1984). Smoking may be directly toxic to the ovaries, and decreased estrogen production not reversible upon abstinence from cigarettes.

Another possible peripheral mechanism of low estrogen production in women may simply reflect their lower body fat. With less adipose tissue, less peripheral aromatization of androgens to estrogens occurs; it has already been discussed that adipose tissue is the most important site in humans for this process and that degree of adiposity is proportional to estrogen levels. With respect to Chapter 1, it is clear that both male and female smokers, as a group, weigh less and are less fat than their nonsmoking peers. Thus, it is entirely plausible that low estrogen levels in female smokers are, at least in part, due to low adiposity.

Clearly, regardless of the mechanism, be it ovarian, peripheral, or both, females who smoke demonstrate a definite anti-estrogenic effect.

Men

While there is considerable evidence for an anti-estrogenic effect of smoking in women, the situation for male smokers clearly differs. Ironically, available evidence reveals that males who smoke have *greater* levels of circulating estrogens when compared to nonsmoking males (Klaiber & Broverman, 1988; Stefanick et al., 1987; Barrett-Connor & Khaw, 1987; Klaiber et al., 1984; Lindholm et al., 1982; Michnovicz et al., 1989) and lower levels of SHBG (Stefanick et al., 1987). Only one study has shown no significant association between smoking and estrogens in men (Dai et al., 1988). Since smoking appears to increase relative androgenic/estrogenic activity in women and decrease relative androgenic/estrogenic activity in men, it seems that smoking, like obesity, contributes towards a convergent morphology of the sexes by altering hormonal profiles. At this point

it is worth noting that the WHR correlates positively with androgenicity in women, but negatively with androgenicity in men (Wahby et al., 1989).

Considering the positive association between estrogens and body fatness, it is not clear why male smokers, with increased estrogen levels, weigh less and are less fat than their nonsmoking counterparts. The situation is even more confusing in view of results of a study indicating that smoking may suppress testosterone levels; male smokers who abstained from tobacco *increased* testosterone levels (Briggs, 1973). Having noted that smokers of either sex tend to gain weight and increase body fatness upon cessation of smoking, it is possible that in women this is due to an increase in estrogens, but this argument does not hold for men in whom a decrease in estrogens and increase in testosterone would be expected to decrease, not increase, body fatness. The following may explain this apparent paradox: male smokers could represent a specific population that is stable at a "reduced weight" because significantly elevated adipose tissue LPL activity (for relative weight) has been found in male smokers (Brunzell et al., 1980). Interestingly, a similar elevation of adipose tissue LPL activity has been found in previously obese persons who were able to remain weight stable at a reduced weight by controlling their energy intake (Schwartz & Brunzell, 1978). No similar finding has been made in females.

Relationship of Smoking to Regional Adiposity

As stated, the inverse relationship of smoking to obesity has been studied extensively. Biological mechanisms such as LPL activity in adipose tissue (Camey & Goldberg, 1984; Brunzell et al., 1980) and effects on 24-hour energy expenditure (Hofstetter et al., 1986; Stamford et al., 1986) have been proposed to explain this association, as also have behavioral mechanisms such as effects on appetite (Elgerot, 1978) and food preferences (Byrd et al., 1988).

There have been very few studies, however, of the association between body fat distribution and smoking. Only a small number of cross-sectional studies have related cigarette smoking to the pattern of fat distribution, and no longitudinal studies have been reported in this area. Various forms of fat distribution indices have been utilized in examining the effects of smoking on regional adiposity.

Of the seven reported studies utilizing the WHR as an index of fat distribution, all showed a significant positive association between the WHR and smoking. One of these studies specifically concerned premenopausal females (Tonkelaar et al., 1989), another postmenopausal females (Kaye et al., 1990), three both males and females (Haffner et al., 1986a; Cox, 1989; Barrett-Connor & Khaw, 1989), and the last two males only (Shimokata et al., 1989; Selby et al., 1990) (the latter consisting of 265 pairs of

monozygous and dizygous twins). Similarly, the ratio of waist girth to hip breadth (WHbR) has been shown to be significantly and positively related to cigarette smoking in men (Troisi et al., 1990), but effects of smoking are inconsistent on another index of fat distribution, the subscapular and triceps skinfold ratio. For males (Selby et al., 1990) and both males and females (Haffner et al., 1986a), there appears no correlation between smoking and skinfold ratios, but a longitudinal study of subjects who stopped smoking showed a marked increase in subscapular skinfold thicknesses, with no change in the triceps skinfold (Comstock & Stone, 1972). Because the WHR and skinfold ratios are only weakly related to each other (Haffner et al., 1986a), it has been speculated that cigarette smoking may very well have differential effects on these indices of the pattern of body fat distribution (Shimokata et al., 1989; Selby et al., 1990). Regardless, it appears that cigarette smoking effects shifts in body fat topography toward the android distribution -- primarily by affecting waist circumference (Selby et al., 1990) -- thus explaining differences noted with use of the WHR index. At present, the mechanisms that underlie the effects of smoking on the pattern of fat distribution are not known and continue to remain obscure (Shimokata et al., 1989; Tonkelaar et al., 1989; Selby et al., 1990).

Other Hormonal Effects of Smoking: Implications for Fat Distribution

There is evidence indicating that smoking has other hormonal effects which may relate to adipose tissue distribution in women. Basically, these effects can be split into two categories: those promoting androgenic activity, and those promoting cortisol production; both derive from the fact that smoking stimulates secretion of adrenocorticotropin (ACTH) (Seyler et al., 1986; Fuxe et al., 1989), with consequent effects on the adrenal glands. Admittedly, effects of smoking on adrenal androgens have been discussed already, but the following considers the effect of smoking on other hormones which may indirectly mediate changes in androgenic/estrogenic activity. The degree to which primary effects of smoking on androgen/estrogen balance are mediated by these other effects is currently unclear. Cortisol is included in this discussion because it is (a) affected by smoking and (b) implicated in body fat distribution. Moreover, hyperandrogenism tends to be associated with hypercortisolism and these factors together are associated with android adiposity (Vague et al., 1985; Mori, 1989). But the extent to which cortisol influences adipose tissue distribution has not been thoroughly investigated, and rigorous longitudinal studies of its involvement remain to be conducted (Rivera & Svec, 1989).

Endogenous Opiates and Prolactin

Serum levels of ACTH are paralleled in a wide range of conditions (including smoking) by prolactin, ß-lipotropin (ß-LPH) and ß-endorphin (Robyn & Tukumbane, 1983; Seyler et al., 1986; Meyerhoff et al., 1988; Mutti et al., 1989). Both nicotine and cigarette smoke have been found to induce secretion and release of ß-LPH and ß-endorphin (Fuxe et al., 1989; Read, 1984; Chernick, 1983; Tobin et al., 1982). Elevations of ß-LPH and ß-endorphin are associated with some forms of female hyperandrogenism (Ruutiainen et al., 1985; Givens et al., 1980), where basal levels of androstenedione, testosterone, 17α-hydroxyprogesterone and LH are typically elevated. Smoking might thus predispose females toward a more androgenic profile by elevating endorphin levels, but how would endorphins mediate this effect?

The answer to the above question appears to involve prolactin -- a pituitary hormone suggested as being androgen-stimulating (Vermeulen et al., 1977; Vermeulen & Ando, 1978), working in conjunction with ACTH. Endogenous opiates are known prolactin releasers (Vanvugt et al., 1989; Robyn & Tukumbane, 1983), and there are significant relationships between high concentrations of prolactin, high concentrations of androgens, and low concentrations of estrogens in women (Grinsted et al., 1989). Furthermore, it has been suggested that prolactin-mediated stimulation of adrenal androgen production is the mechanism by which ovulation is suppressed in polycystic ovarian syndrome and Cushing's syndrome (Robyn & Tukumbane, 1983). But whether smoking increases (Klevene & Balossi, 1986; Seyler et al., 1986; Gossain et al., 1986) or decreases (Andersen A.N. et al., 1984; Andersson et al., 1985a; Baron et al., 1986; Andersson et al., 1988) prolactin levels is currently unclear, perhaps because the effect of smoking on prolactin is secondary to a primary effect on endorphins. Nonetheless, the suggestion that smoking-induced endorphin-mediated elevations in prolactin levels might influence body fat distribution is supported by the observation of a significant correlation between WHR and prolactin concentrations in women (Grenman et al., 1986). Prolactin furthermore alters the lipolytic response of adipose tissue to norepinephrine and increases LPL activity (Vernon & Finley, 1985; Zammit, 1985), but regional variations in response remain to be investigated.

Given that prolactin is considered to be androgen-stimulating (Robyn & Tukumbane, 1983), it remains to be elucidated whether prolactin affects specifically the adrenals, ovaries, or both. The adrenal glands have the highest prolactin-binding activity of all tissues in the body (Shiu & Friesen, 1974), and hyperprolactinemia is associated with elevated serum DHEA-S (primarily reflecting increased DHEA-S production rate) (Schiebinger et al., 1986). But adrenal production of androstenedione and testosterone has

been reported to be either increased or unchanged by prolactin stimulation (Robyn & Tukumbane, 1983). Hyperprolactinemic women, however, have elevated circulating levels of dihydrotestosterone (DHT) (Giusti et al., 1978), suggesting that prolactin influences the activity of 5α-reductase, the membrane-bound enzyme which converts testosterone to DHT. Several findings confirm the positive influence of prolactin on 5α-reductase activity in the ovaries (Polan & Behrman, 1981), gonads (Baranao et al., 1981) and liver (Lax et al., 1976).

Cortisol

Secretion of cortisol from the adrenals is controlled by ACTH, and elevations of cortisol in association with smoking and/or nicotine exposure are consistent with smoking-induced increases in ACTH. Habitual smokers display significantly elevated plasma cortisol precursor levels (Friedman et al., 1987), and plasma cortisol levels are also significantly increased after smoking (Andersson et al., 1985b; Gossain et al., 1986; Targovnik, 1989). But when nonsmokers and smokers *both* smoke cigarettes, increases in plasma cortisol are significantly greater in the nonsmokers than in the smokers (Sellini et al., 1989a; Sellini et al., 1989b). Allowing development of a certain degree of tolerance, chronic smoking still appears to elevate plasma cortisol levels, but this has not been shown to be reflected by measures of 24-hour urinary-free cortisol levels (Yeh & Barbieri, 1989). It is interesting to note that in contrast to almost immediate elevations of ACTH, ß-LPH, ß-endorphin and prolactin after stimulation, increases in plasma cortisol take two to three times as long to occur (Meyerhoff et al., 1988). Furthermore, it has been put forth that the initial effects of nicotine on these same hormones (that is, hypersecretion) eventually disappear, to be replaced by maintained hypersecretion of cortisol only (Fuxe et al., 1989).

Elevated levels of cortisol are conducive to the development and maintenance of android adiposity, which provides further basis for the association of smoking with centrally-localized body fat. Hypercortisolism from either endogenous secretion or exogenous administration of glucocorticoids can result in the clinical manifestations of Cushing's syndrome, one characteristic of which is central body fat (Griffing & Melby, 1983; Dhein, 1986). As assessed by computed tomography (CT), subjects with true Cushing's syndrome have higher levels of subcutaneous abdominal fat by a factor of two and greater levels of intraabdominal (visceral) fat by a factor of five in comparison to normal subjects (Mayo-Smith et al., 1989). Also as assessed by CT, subjects on extended glucocorticoid therapy display higher mediastinal (deep) fat areas in comparison with normal subjects (Horber et al., 1986). These results confirm earlier reports of the effect of chronic elevations of endogenous or exogenous glucocorticoids on android adiposity

(Vague et al., 1967a; Vague et al., 1969). It would appear that these effects of cortisol are *direct;* specific binding of corticosteroid hormones occurs in human adipose tissue, an effect which is regionally specific (Rebuffé-Scrive et al., 1985b).

Android obesity *per se* is associated with a minor degree of hypercortisolism; relationships have been established between cortisol production rates and masculine characteristics of fat distribution (Vague et al., 1967b; Vague et al., 1969). As with prolactin, cortisol levels also correlate (albeit weakly) with WHR (Hauner et al., 1988), and significantly higher concentrations of cortisol have been found in abdominal adipose tissue in comparison to that from other areas (Newton et al., 1986). Notably, the highest glucocorticoid receptor density is found in intra-abdominal, as contrasted with subcutaneous abdominal, tissue; there are, however, no differences in receptor affinities (Rebuffé-Scrive et al., 1985b). In keeping with this latter finding and concerning expression of glucocorticoid receptors in regional fat depots, mRNA concentration has been found to be highest in omental fat tissue, followed by subcutaneous abdominal and femoral fat depots (Rebuffé-Scrive et al., 1988b). Not surprisingly, administration of glucocorticoid antagonists (acting at the receptor level) ameliorates the central adiposity of Cushing's syndrome (Nieman et al., 1985).

Cortisol also appears to have regionally-specific effects on adipocyte metabolism which promote android adiposity. Young women with Cushing's syndrome demonstrate considerably greater LPL activity in the abdominal region in comparison to control subjects (Rebuffé-Scrive et al., 1988a), and this explains in part the large abdominal adipocytes by which this condition is characterized. In rats, although effects of corticosteroids on LPL activity are unclear (de Gasquet & Pequignot, 1973; de Gasquet et al., 1975), glucocorticoids do mediate regionally-specific effects upon glucose uptake (Hauner & Pfeiffer, 1989). Norepinephrine-stimulated lipolysis in the abdominal region is low in women with Cushing's syndrome (Rebuffé-Scrive et al., 1988a), and women exposed to exogenous corticosteroids have been found to have smaller gluteal adipocytes than control subjects, as well as smaller gluteal skinfold thicknesses (Krotkiewski et al., 1976). These observations are consistent with the commonly-observed peripheral to central redistribution of fat seen with elevated levels of adrenal corticosteroids. Moreover, regional differences in lipolysis after administration of glucocorticoids have been observed in rat adipose tissue; lipolytic response of subcutaneous adipocytes is decreased (Hauner & Pfeiffer, 1989).

Finally, it is possible that cortisol could promote abdominal adipocyte hyperplasia: *in vivo* (Hauner et al., 1987) and *in vitro* (Hauner et al., 1989) differentiation of human adipocyte precursors into mature adipocytes has been shown to be triggered by a combination of cortisol and insulin, and this can occur in adults (Hauner et al., 1989).

Notably, the effects of cortisol appear to be independent of sex steroids but dependent upon insulin (Hauner et al., 1989).

Summary

This section has reviewed the extent of the known effects of smoking in altering relative androgenic/estrogenic balance, finding considerable evidence for an anti-estrogenic and/or pro-androgenic effect of smoking in women. Compared to nonsmoking women, females who smoke display an earlier age of natural menopause, decreased fertility, greater risk for development of osteoporosis and oligomenorrhea, lower rates of estrogen-dependent cancers and lower levels of estrogens and/or increased levels of androgens. It is clear that females who smoke display a greater degree of relative androgenic/estrogenic activity. The mechanisms of this effect are not clear; lower body fat or aromatase inhibition resulting in less peripheral aromatization, increased hepatic estrogen degradation, or induction of microsomal oxidative systems are all possible peripheral/extraovarian explanations. Decreased ovarian production of estrogens by aromatase inhibition of granulosa cell function or other directly toxic effects are conceivable at the central/primary level. It is also possible that interactive effects of smoking with other hormones such as endorphins and prolactin might accentuate an increase in relative androgenic activity; such an effect could additionally suppress estrogen production. Male smokers clearly display greater endogenous estrogen levels than nonsmokers, but it is unclear how and why this occurs. Smokers of either sex display elevated levels of cortisol, and cortisol is related to and involved in the promotion of android adiposity. This involvement of cortisol does not appear as important as that of the sex steroids, although further research is required to clarify this contention. Both male and female smokers weigh less and are less fat than their nonsmoking counterparts, and there are significant associations of smoking with abdominal (android) distribution of body fat in either sex.

Conclusion

Based on evidence presented and discussed in this chapter, there is a substantial basis for the two-part hypothesis that: (a) the estrogens are directly involved in determining and maintaining the "healthy" gynoid adipose tissue distribution; and (b) premenopausal females who smoke display greater androgenic relative to estrogenic activity, and that this is associated with "unhealthy" android adiposity. These two factors taken together imply that the mechanism by which smoking is associated with android adiposity concerns an

alteration of hormonal balance in premenopausal women. Further research should be directed towards examining the interaction between androgen/estrogen balance, smoking and regional adipose tissue distribution in premenopausal women, with specific consideration of the health risks associated with android adiposity.

CHAPTER 3: METHODS AND PROCEDURES

Subjects

Recruitment

Subjects were recruited by means of bulletins posted in local health clinics, physicians' offices, hospitals, life insurance companies, government offices, health clubs, universities and colleges. Bulletins enabled self-selection by provision of eligible gender (female), age range (20-35 years) and smoking status (smoker/nonsmoker). Subjects were also recruited via public service announcements carried by local television and radio stations. Some local stations carried the study as a news item, and the author participated in several live television and radio interviews and talk shows which covered the background area and raised public awareness. An additional source of subjects was by the recommendation of individuals already participating in the study. Subjects were promised a complete set of their personal results in exchange for participation.

Upon expressing interest in the study, individuals were mailed an information package and consent form (Appendix B). Having thus been informed in writing of (a) the purpose of the study (b) the nature and extent of their prospective participation (c) explanations of all testing procedures (d) potential risks and discomforts involved (e) the name, phone number and address of supervisory personnel in the event of any questions or complaints and (f) their right to deny consent and/or withdraw from the study at any time without prejudice, subjects then provided their written, voluntary consent and completed a screening questionnaire (Appendix C). The screening questionnaire assessed, by self-report, the status of seven categorical variables (smoking or smoke exposure, oral contraceptive use, alcohol consumption, acute weight change, physical activity, parity and menstrual cycle regularity). All forms were returned by mail to the author who then contacted subjects by telephone regarding their further participation. It should be noted that in an essentially observational study such as this, where no experimental manipulation of variables occurs, groups must naturally be formed according to the inherent properties of individuals. Random sampling and random allocation to treatment groups was not feasible.

Selection

An initial number of 187 consent forms and screening questionnaires were mailed out to prospective participants. Return rate was 71.7 % (53 non-returns); one hundred thirty four (134) adult females were thus screened via the self-reported questionnaire

(Appendix C). Any unclear responses were clarified by telephone. On the basis of considerations outlined in Chapter 1, and to decrease random variation of results, exclusion criteria included: (a) age less than 20 or greater than 35 years; (b) history of oral contraceptive use or any other hormonal medication (estrogens, progesterone, glucocorticoids, or thyroid) within four months prior to participation in the study; (c) abnormal cyclical menstrual history; (d) hirsutism; (e) pregnancy; (f) prescription or non-prescription drug use within one month of participation in the study; (g) prior history of any endocrine abnormality; (h) fluctuations in weight of 25 lb. or more within the six months preceding the study; and (i) competitive athleticism and/or intense (subject-rated) activity and/or greater than eight hours of planned physical activity per week. Failure to meet these criteria resulted in 52 subjects being dropped from the sample. The major reason for exclusion was oral contraceptive use.

The sample was then further subdivided on the basis of self-reported smoking status. Of those remaining, 46 were nonsmokers and 36 were regular cigarette smokers. In view of previous research involving smoking status and body weight (Albanes et al., 1987), only smokers reporting a minimum of eight cigarettes per day for the five year period prior to participation in the study were included in the sample. Intermittent smokers reporting seven cigarettes per day or less were thus dropped from the sample (n=9). Nonsmokers were defined as those who had never smoked ("never" smokers) and/or former smokers who had: (a) not smoked at all for at least the five year period preceding participation in the study; and (b) not smoked for a period of time equal to or greater than the duration of the period for which they smoked. Former smokers who did not meet these criteria (n=17) were dropped from the sample, leaving the "nonsmoking" group composed of 16 never smokers and 13 former smokers. Passive exposure to smoke was also considered as a basis for exclusion from the nonsmoking group in view of evidence indicating that nonsmokers so exposed display significantly altered hormone levels (Sellini et al., 1989c), but questionnaire responses did not reach beyond "moderate" degrees of exposure for three hours once per week. Regarding the decision allowing former smokers to qualify as nonsmokers: this was made on the basis of previous research indicating significant differences in fat distribution (waist girth and WHR) between current smokers and both never *and* former smokers, with no significant differences between never smokers and former smokers (Shimokata et al., 1989). Final sample size was therefore 56 (27 smokers, 29 nonsmokers), this being in excess of the number (50) required by calculations of statistical power (Appendix A). Fifty-three women were white, and three women were black (two smokers, one nonsmoker); it was hypothesized that inclusion of blacks in the sample would not influence group means to any great extent since ethnicity has been

demonstrated to account for only 4.8% of the variance in fat distribution in women (Haffner et al., 1986a). Four subjects (all white; two smokers, two nonsmokers) failed to complete the study after completing the first (anthropometric) series of measurements; their results were included in descriptive statistics of groups and in analyses of fat distribution but, because no hormone values were available, their results are missing from analyses of sex hormone levels and analyses of interactive effects of smoking with hormone levels and fat distribution. Of these subjects, one became pregnant, two became amenorrheic and one could not be available at the required point of her menstrual cycle so that her blood could be drawn. Average age of participants was 29.25 ± 4.45 years ($\bar{x} \pm$ SD) (range 20-35 years). For smokers, the average number of cigarettes smoked per day was 16.9 ± 6.3 ($\bar{x} \pm$ SD) (range 8-30 cigarettes); the average number of years smoked for was 12.9 ± 4.5 ($\bar{x} \pm$ SD) (range 5-21 years); and the average age of initiation of smoking was 16.9 ± 2.9 years ($\bar{x} \pm$ SD).

Research protocol was approved by the appropriate University of Manitoba Committee on Research Involving Human Subjects (both Faculty of Medicine and Faculty of Physical Education and Recreation Studies). This protocol also included assessment of total body and site-specific bone mineral density (Ward's Triangle, femoral neck, greater trochanter and lumbar spine) by dual X-ray absorptiometry (Lunar DPX). In addition, estimates of percent body fat based on assessment of total body density by X-ray absorptiometry were made (utilizing software provided by Lunar Corporation); these estimates are reported in Chapter 4 as descriptive information. However, bone mineral density results will be reported elsewhere. This thesis concerns sex hormone levels and adipose tissue distribution only. Data collection ran April through September, 1990.

Experimental Design

The study was cross-sectional and fundamentally descriptive. There was no true manipulation of the independent variable (smoking) upon the dependent variables (sex hormone levels and body fat distribution). Instead, status of the dependent variables was observed in each of the smoking and nonsmoking groups and data analyzed for differences between groups. Dependent variables indicative of relative androgenic/estrogenic activity have been previously mentioned. These were total serum estradiol, total serum testosterone, the testosterone/estradiol ratio, the "free" (non-SHBG bound) portions of these hormones and SHBG. The primary dependent variable indicating body fat

distribution (android/gynoid) was WHR, but other girth ratios indicative of fat distribution were also considered.

The possibly confounding effects of certain *categorical* variables on the dependent variables were controlled by screening them out. These variables were acute weight change, excess physical activity, oral contraceptive or other hormone use and menstrual cycle irregularities or abnormalities. In this situation these categorical variables were effectively also control variables.

A *continuous* variable which had potential to confound observations of sex hormone levels and body fat distribution was total adiposity. With respect to Chapter 1, it was concluded that the likely interaction between total degree of adiposity and regional adipose tissue distribution may best be controlled by adjusting for total degree of adiposity. Chapter 2, Section I, illustrated the positive association between sex hormone levels and total adiposity. This interactive effect may also best be controlled by adjusting for total degree of adiposity. Therefore, the confounding effects of total degree of adiposity on both sets of dependent variables were controlled by treating overall adiposity as a covariate. It was thought that controlling for categorical or continuous variables respectively by either screening or adjusting would increase the sensitivity of the study by decreasing overall "error" variance (that unaccounted for).

Extraneous variables, these being variables beyond control, were limited. The major extraneous variables were inter- and intra-individual variability in menstrual cycle timing; these had potential to influence the variability of sex hormone levels even though specimen collection was standardized at a certain point of the menstrual cycle.

Procedures

Sex Hormone Profiles

Blood Samples

Ten milliliters (10 mL) of blood was drawn into a glass serum separator tube by venipuncture of the antecubital vein between 0800 and 1200 hours following an overnight fast of at least 10 hours and abstinence from alcohol for at least five days. Blood was drawn in the mid-follicular phase (on the seventh or eighth day of the menstrual cycle) by a Registered Technologist of Nuclear Medicine in the Department of Nuclear Medicine, St. Boniface General Hospital, 409 Tache Avenue, Winnipeg. It was at this point that percent body fat, total body and site-specific bone mineral density were determined by dual X-ray absorptiometry but, as noted, the bone density results will be reported elsewhere. Blood

samples were refrigerated for no more than three hours, then transported on ice to the Endocrinology and Metabolism Laboratory, Section of Endocrinology and Metabolism, Health Sciences Centre, 700 William Avenue, Winnipeg. After the blood stood at room temperature for approximately one hour, serum was obtained by centrifugation at 3300 rpm for 15 min (room temperature). A serum plasma separator was utilized (Sure-Sep Ltd.). Aliquots of obtained serum were stored on location at -20° C until analyzed. It should be noted that for several of the subjects (n=6), the seventh or eighth day of their menstrual cycle coincided with a weekend, necessitating the bone scan and blood withdrawl at St. Boniface General Hospital on a Saturday. Because the Endocrinology and Metabolism Laboratory was not open on weekends, blood was instead centrifuged and serum obtained according to the above description at St. Boniface General Hospital, where aliquots of serum were then frozen at -20° C until being transported on dry ice to the Endocrinology and Metabolism Laboratory during regular working hours for storage and later analysis. All samples were identified only by subject identity number, the author's name, and the date and time of collection; subjects' name and smoking status were not noted.

Analytical Methodology

All blood analyses were performed on serum specimens at the Endocrinology and Metabolism Laboratory, Health Sciences Centre, Winnipeg. The author assisted in analyses under direction of the Laboratory Director. Total serum testosterone concentration was determined by commercially purchased solid phase ^{125}I-radioimmunoassay (RIA) kits [Coat-a-Count®; DPC Diagnostic Products Corp., Los Angeles, CA 90045; lot numbers: TTT1323 (antibody-coated tubes), TTT20284 (^{125}I), TTC3030 (standards)]. Total serum SHBG and estradiol concentrations were determined by commercially purchased solid phase fluoroimmunoassay (FIA) kits [DELFIA®; Wallac Oy, Turku, Finland SF-20101; SHBG kit no. 1244-025, lot no. 608551; estradiol kit no. 1244-043, lot no. 608461; standards, europium tracers, antisera, assay buffers and microtitration strips prepared by Farmos Diagnostica, Oulunsalo, Finland]. There was one assay series for testosterone, and two series each for SHBG and estradiol; an equal number of samples from each of the smoking and nonsmoking groups were included for each of the latter two assay series. All assays were performed in duplicate, and individual assays were monitored by quality control samples provided with each kit. Accuracy in comparison to control sera was within 2%. Female pool samples (provided by the Endocrinology and Metabolism Laboratory) were within normal ranges. Intra-assay coefficient of variation for testosterone was within 4%. Intra-assay coefficients of variation were within 6% for SHBG and within 3% for estradiol. Inter-assay coefficients of variation for SHBG and estradiol were both ~8%.

There were 36 samples in the first, and 16 samples in the second, assays for both SHBG and estradiol.

Testosterone.

The procedure for RIA of testosterone is based upon a testosterone-specific antibody immobilized to the wall of a polypropylene tube. A Cavro diluter was used to transfer duplicate 50μL volumes of standards, quality control samples and subjects' sera with ^{125}I-testosterone to numbered, antibody-coated tubes. The tubes were mixed well and allowed to incubate for two hours, during which time the ^{125}I-labelled testosterone competed with testosterone in the subjects' samples and standards for antibody sites. The tubes were then decanted, which allowed separation of the bound from the free testosterone. The levels of radioactivity were counted, these being inversely proportional to the concentration of testosterone in the subjects' sera.

Estradiol.

The procedure for FIA of estradiol is based upon competition between europium-labelled estradiol and sample estradiol for polyclonal anti-estradiol antibodies (derived from rabbit) which are coated to microtitration strips (in strip wells). Estradiol in standards, controls and subjects' sera inhibit the binding of the europium-labelled estradiol to the antibody molecules. A second antibody, directed against rabbit IgG, is coated to the solid phase, and binds the IgG-estradiol complex, which provides separation of antibody-bound and free antigen. After washing the required number of microtitration strips, 25μL of estradiol standards and subjects' sera was pipetted into the strip wells. To each well was added 100μL of diluted estradiol antiserum solution. The microtitration strips, in a strip frame, were incubated slowly on a plate shaker at room temperature for 30 minutes, after which 100μL of diluted europium-labelled estradiol was pipetted into each well. The frame was again incubated at room temperature for two hours with slow shaking on a plate shaker. After incubation, each strip was then aspirated and washed, and 200μL of Enhancement Solution dispensed into each well. The Enhancement Solution dissociates europium ions (Eu^{3+}) from the labelled estradiol into solution, where they form highly fluorescent chelates with components of the Enhancement Solution. The frame was shaken slowly for five minutes, then 15 minutes allowed to elapse before measuring fluorescence in a time-resolved fluorometer, the amount of light emitted from each sample being directly proportional to the concentration of estradiol in the sample.

SHBG.

The dissociation-enhanced lanthanide fluoroimmunoassay (DELFIA) of SHBG is a solid phase, two-site fluoroimmunometric assay based on a direct sandwich technique in which polyclonal anti-SHBG antibodies (produced in a rabbit) and monoclonal antibodies

(produced in a mouse) are used. In a one-step procedure, diluted standard, control and subjects' samples were reacted simultaneously with polyclonal anti-SHBG antibodies immobilized on the inside walls of plastic microtitration strip wells and europium-labelled monoclonal antibodies in solution. After washing, Enhancement Solution was added to dissociate Eu^{3+} cations from the labelled antibody into solution, where they formed highly fluorescent chelates with components of the Enhancement Solution. Fluorescence in the microtitration strip wells was measured in a time-resolved fluorometer (as with estradiol). The set of SHBG standards was used to plot a standard curve of fluorescence intensity versus SHBG concentration from which the concentration of SHBG in subjects' specimens was calculated.

Instrumentation

RIA samples for total serum testosterone were counted in an LKB Wallac 1260 MULTIGAMMA® Gamma Counter. FIA samples for SHBG and estradiol were counted in an LKB Wallac 1230 ARCUS® Fluorometer.

Anthropometric Measurements

All measures were performed by the author at the Sport & Exercise Sciences Research Institute, Fort Gary Campus, University of Manitoba. Prior to embarking upon this study, the author had received training in anthropometric measurement according to protocol of the International Society for the Advancement of Kinanthropometry (ISAK). Work proceeded according to a detailed proforma form (Appendix D), where strategic arrangement of measures and sites facilitated systematic data collection. Subjects were identified by name, sex, smoking status and code number. Date of birth and measurement date were recorded. Subjects wore swimwear or similar clothing, and were in a post-absorptive state. Where possible, subjects voided prior to commencement of the anthropometric procedures. Subjects were advised in advance to avoid excessive sweating, ingestion of large quantities of water, or very salty foods prior to measurement; it was emphasized that a "normal" state of hydration was required for valid assessment of weight, girths and skinfold thicknesses. All measures were performed in triplicate and the median value taken as the "true" value. While the mean value may be thought to be the most representative, anthropometric errors can sometimes be relatively large; hence use of the median value, which is less influenced than the mean by such errors. By convention, all subjects were assessed in the "standard anatomical position"; the subject stood with eyes and head pointing forward, upper limbs by the sides with palms forward, thumbs directed

away from the sides with fingers pointing directly downward and the feet together with the toes pointing directly forward.

In addition to measurement of girths, weight, and height, hip breadth and several skinfold measurements were also required. Hip breadth was included in the protocol to check for any differences between groups in pelvic structure which may have confounded interpretation of girth ratios. Skinfold thicknesses were assessed with the intention of adjusting measures of regional adiposity and sex hormone levels for a comprehensive sum of skinfolds, since both sets of variables appear related to overall adiposity. Differences in body size were accounted for by adjusting sums of skinfolds for size, taking height to be an indicator of size. It is important to realize that relative fatness varies with body size; most investigations simply do not address the validity of comparing skinfold thicknesses across a wide spectrum of sizes. Sums of skinfolds were multiplied by the ratio of subject stature (with a dimensional exponent of one) to a specified "Phantom" stature (Ross W.D. & Ward, 1986). This reference stature is entirely arbitrary; the Phantom is simply a single, unisex reference human utilized as a calculation device for quantifying proportional differences (Ross W.D. & Ward, 1982). Insofar as this model makes use of the geometrical similarity system, an inherent assumption is that geometrical similarity holds when proportional skinfold adjustments are made. However, an adjustment for height is clearly better than no adjustment at all.

It should be noted that estimation of overall adiposity by sum of skinfolds was thought to be superior to use of the BMI or estimation of percent fat from skinfold thicknesses. BMI is clearly invalid for individual or small group comparisons, and prediction of percent fat by skinfold caliper requires many assumptions, none of which have ever been validated (Martin et al., 1985). Prediction of percent fat from densitometry by underwater weighing was a possible method, one which is more "direct" than the skinfold caliper method, but even hydrostatic densitometry requires assumptions that have never been validated (Martin et al., 1985). Likewise with estimates of percent fat from X-ray absorptiometry, but these data were available and are reported for completeness and descriptive interest only. The primary and most valid indicator of overall adiposity in this study is considered to be the sum of skinfolds; it is not predicated on assumptions which inevitably increase error variation. Admittedly, inclusion of internal fat in the estimation of overall adiposity by sum of skinfolds was not possible, but most of the fat of the human body is subcutaneous, and the age range of the sample group (20-35 years) allows speculation that internal fat, at least in this specific case, was not of great concern.

In the interest of contributing to an anthropometric database, measurements in excess of those required for this particular study were taken. Only those procedures

directly applicable to this study are described in the following text; they are those of Ross W.D. and Marfell-Jones (1982a). For information on other measures noted on the Anthropometric Profoma Form (Appendix D), the interested reader is referred to Ross W.D. and Marfell-Jones (1982a).

Girths and Hip Breadth

With respect to Chapter 1 -- Assessment of Regional Adiposity, minimum *waist* circumference was taken where the waist was best defined, approximately halfway between the costal border and iliac crest. Where a well-defined waist was not apparent, the circumference was taken halfway between the manubrium sterni and umbilicus. An *umbilical* (abdominal) circumference measurement was also taken at the level of the umbilicus. Measurement of both the waist and umbilical circumferences were following a normal expiration (end-tidal). Maximum *hip* (gluteal) circumference was obtained at the level of the greatest posterior protuberance. This measure was taken with the subject erect, feet together. A *thigh* circumference measurement was obtained at a level of one-to-two centimeters below the gluteal line or at the arbitrary join of the gluteal muscle protuberance with the thigh; the subject stood erect with feet slightly parted and weight evenly distributed for this measure. For all circumference measurements the tape was in contact with the skin but did not compress underlying tissues.

Hip (bi-iliac) *breadth* was taken as the distance between the most lateral points on the superior border of the iliac crest. Branches of an anthropometer were utilized as a sliding caliper which was applied upwards at an angle of about 45° from the horizontal to encompass the greatest diameter between the lateral aspects of the iliac crests. Firm pressure was applied to the branches over the iliac sites with the index fingers so as to obtain a "true" value for the bony breadth independent of overlaying adipose tissue.

Skinfolds

Thickness measurements were taken on the right side at the following defined sites. For each subject, a central (iliac crest, supraspinale and abdominal) and overall sum of skinfolds was calculated; each respective sum was then adjusted for height.

Biceps.

The caliper reading when applied one centimeter distally from the left thumb and index finger raising a vertical fold at the mid-acromiale-radiale line on the anterior surface of the arm.

Triceps.

The caliper reading when applied one centimeter from the left thumb and index finger raising a vertical fold at the mid-acromiale-radiale line on the posterior surface of the arm.

Subscapular.

The caliper reading when applied one centimeter distally from the left thumb and index finger raising a fold beneath the inferior angle of the scapula in a direction running obliquely downwards at an angle about 45° from the horizontal.

Iliac Crest.

The caliper reading when applied one centimeter anteriorally from the left thumb and index finger raising a fold immediately superior to the iliac crest at the mid-axillary line.

Supraspinale.

The caliper reading when applied one centimeter anteriorally from the left thumb and index finger raising a fold about seven centimeters above the spinale on a line to the anterior axillary border. The fold follows the natural fold lines running medially downwards at about a 45° angle from the horizontal.

Abdominal.

The caliper reading when applied one centimeter inferior to the left thumb and index finger raising a vertical fold which is 5 cm lateral to and at the level of the mid-point of the navel.

Front Thigh.

The caliper reading when applied one centimeter distally to the left thumb and index finger raising a fold on the anterior of the right femur when the leg is flexed at an angle of 90° at the knee by placing the foot on a box. The mid-thigh position for this measure is the estimated half-distance between the inguinal crease and anterior patella.

Medial Calf.

The caliper reading when applied one centimeter distal from the left thumb and index finger raising a vertical fold on the medial right calf at the estimated greatest circumference with the leg flexed to an angle of 90° by placing the foot on a box.

Weight

Weight determination was made on a calibrated digital electronic weigh scale with measurement made to the nearest 0.05 kg.

Height

The subject stood erect and barefoot against a vertical wall with heels together and arms relaxed against the sides. The measurement was taken as the maximum distance from the floor to the vertex of the head. The vertex is the highest point on the skull when the head is held in the Frankfort Plane, which is the position where an imaginary line joining the orbitale (most inferior position on the margin of the eye socket) to the tragion (notch superior to the flap of the ear at the superior aspect of the zygomatic bone) is horizontal.

Instrumentation

Girths were measured with a Lufkin® Executive flexible (6.5mm wide) steel tape calibrated in centimeters with millimeter graduations (model W606PM). The tape was non-extensible with a stub before the zero line. Accuracy of scale was checked at the commencement of measuring procedures.

Skinfold thickness measurements were determined by use of a Harpenden caliper (British Indicators Ltd., Acrewood Way, Hatfield Road, St. Albans, Herts, England). Calibration was checked by fixing the caliper and suspending weights from the lower jaw. Skinfold thickness measurements were facilitated where required by use of an anthropometric box (50 x 40 x 30 cm) for posing the subject.

Breadths were assessed with a GPM anthropometer calibrated in centimeters with millimeter graduations.

Height was assessed with use of a triangular head board forming a right angle from the wall to the vertex of the skull. A mark was scribed underneath and the distance taken from a vertically placed rule in centimeters.

Weight was determined using a Digi digital electronic weigh scale utilizing multiple force transducers. Units were kilograms.

Reliability, Validity and Objectivity

Reliability

There were several factors which may have affected the reliability of measures of sex hormones and regional adiposity. Diurnal variation and timing of the menstrual cycle had potential to influence the observed results of sex hormone assays; however, the time of day and day of the menstrual cycle were standardized (as described), and intra-assay coefficients of variation are reported. Serum samples were analyzed in two batches and, although inter-assay coefficients of variation are reported, the fact that all serum samples were not assayed at once could have affected reliability. However, each batch was made up equally of smokers' samples and nonsmokers' samples. It is acknowledged that assay

reliability may have been affected by varying degrees of compliance with instructions for fasting and abstinence from alcohol.

For anthropometric measures, reliability could have been influenced by many factors. But all variable instruments (skinfold caliper, weigh scale) were calibrated, and anatomical sites were consistently landmarked. Measures for any given site, which were in triplicate, were not taken in succession: the entire set of sites was assessed once, and the process repeated twice. This was especially important in the case of skinfold measures where immediately repeated measurements tend to compress tissue and thus falsely decrease successive readings. Also important was the time allowed for skinfold readings to stabilize -- about two seconds. Procedures were standardized to maximize reliability: the median of the three observed anthropometric values was utilized to avoid errors incurred by averaging, and data collection was systematic. The extent to which subjects complied with respect to a voided, post-absorptive state and "normal" state of hydration could have affected reliability, but any effect was likely of little magnitude.

Given the inverse relationship between reliability and the standard error of measurement (SEM), SEM is reported for dependent variables where relevant.

Validity

There was a high degree of logical validity with respect to all measures described. Use of radioimmunoassay and fluoroimmunoassay to assess sex hormone levels, use of skinfold calipers to assess subcutaneous adipose tissue thickness and use of girth ratios such as WHR to assess body fat distribution all have inherent logical validity. All additionally display high criterion-related validity; for example, radioimmunoassay and fluoroimmunoassay versus column chromatography or extraction, sums of multiple skinfold sites versus total adiposity (this could also be considered an example of content validity), or WHR versus computed tomography. Because most fat in humans is subcutaneous, using adjusted sums of multiple skinfolds to represent total adiposity has construct validity. The content validity of utilizing girth ratios such as WHR to assess regional adiposity may be considered debatable by some (in view of what girths actually assess) but this relates to an acknowledged assumption, besides which, the WHR is the most widely used method for this purpose and its use increased external validity. With respect to subject selection, a broad range of adiposity was allowed. It was hypothesized that this would increase external validity, while adjustment of dependent variables for overall adiposity would increase internal validity.

Objectivity

Objectivity of testing procedures was a primary concern. For both the sex hormone profiles and anthropometric measurements, inter-observer reliability was not an issue; the author and Laboratory Director performed all sex hormone analyses, and the author performed all anthropometric measurements. Hormone analyses were completely unbiased; all samples were coded. Although it was not possible to set up and conduct the anthropometric measurements blind (the author knew which subjects smoked and which did not), this is not viewed as serious limitation or threat to objectivity. Intentional bias was resisted and, although the spectre of unconscious bias cannot be ruled out, any effect was likely to be of little magnitude, considering the relatively gross nature of anthropometric measurement. Blind measurement sessions were considered, but ruled out, since administrative details of the study were completely under the author's control, and such a protocol would have involved others. Ultimately, it was the author's observation that smokers were apparent simply by their odor, a phenomenon noted also, rather wryly, by Garn (1985), who suggested that this was in some way connected to decreased fertility rates. Regardless, intra-observer reliability was a primary consideration; triple measures of anthropometric sites were repeated for any values which appeared to deviate grossly, standards and quality control samples were included in hormone assays, and coefficients of sex hormone assay variation were checked against established laboratory norms.

Statistical Analysis

The primary null hypothesis (H_o) to be tested was: There is no interactive effect of smoking with sex hormone levels or balance and indicators of regional adiposity as demonstrated by comparisons of smoking and nonsmoking premenopausal females. Secondary null hypotheses to be tested were: (a) premenopausal female smokers are not characterized by greater android adiposity than nonsmokers; and (b) premenopausal female smokers are no different than nonsmokers in terms of sex hormone levels or balance. As noted in calculations of subject number (Appendix A), power was 0.80; ß was set at 0.20 so that sample size was not impractically large. Risk of Type II error was 20%. The minimum acceptable level of significance was 0.05; α was therefore 0.05, and the risk of Type I error was 5%.

All data were entered on a Macintosh® SE microcomputer (Apple Computer, Inc.) into Microsoft® Excel (©Microsoft Corporation, 1989) for spreadsheet arrangement and derivation of variables. For anthropometric variables, WHR and umbilical-to-hip girth

ratio (UHR) were computed by dividing hip girth into waist and umbilical girths respectively, and WTR calculated by dividing thigh girth into waist girth. WHbR was derived by dividing waist girth by hip (bi-iliac) breadth. Central sum of skinfolds (SSF_c) was derived by summing the abdominal, supraspinale and iliac crest thicknesses, and overall sum of skinfolds (SSF_o) was derived by summing the biceps, triceps, subscapular, iliac crest, supraspinale, abdominal, front thigh and medial calf thicknesses. For both SSF_c and SSF_o, proportional ($\propto$) adjustments (for height) were made according to the following calculation:

$$\text{proportional } \Sigma \text{ skinfolds} = \text{actual } \Sigma \text{ skinfolds} \times \frac{\text{subject height (cm)}}{170.18 \text{ (cm)}},$$

where 170.18 (5 ft., 7 in.) = an arbitrary Phantom height designed to quantify proportional differences (Ross W.D. & Ward, 1986), thus deriving proportional sum of central skinfolds ($\propto SSF_c$) and proportional sum of overall skinfolds ($\propto SSF_o$). For sex hormone profiles, the T/E_2 ratio was computed by dividing total serum estradiol levels into total serum testosterone levels (both in nmol/L), and the ratios T/SHBG (free androgen index) and E_2/SHBG (free estradiol index) were determined by dividing total serum SHBG into the respective total serum levels of testosterone and estradiol.

To improve kurtosis and skewness, variables representative of regional adipose tissue distribution and sex hormone balance were transformed to logarithmic (base 10) values for analysis. Specifically, anthropometric variables so transformed were WHR, UHR, WTR, WHbR, waist girth, umbilical girth, hip (gluteal) girth, upper thigh girth, $\propto SSF_c$ and $\propto SSF_o$; all hormonal variables were transformed (testosterone, estradiol, T/E_2 ratio, SHBG, T/SHBG and E_2/SHBG). Tests of statistical significance were based on transformed ($\log_{10}$) values; however, the means and standard errors of untransformed values are reported. StatView SE+*Graphics* (©1987 Abacus Concepts, Inc.) and *Super*ANOVA (©1989 Abacus Concepts, Inc.) software was used for statistical analysis of the data.

Descriptive characteristics concerning continuous and categorical differences between groups were appropriately tested by Student's *t* test (independent, two-tailed) and by χ^2 test (one group comparison for parity, contingency table for alcohol consumption). Secondary hypotheses were tested by two-way analysis of covariance (ANCOVA), with proportional sum of overall skinfolds ($\propto SSF_o$) taken to be the covariate. The *Super*ANOVA program includes a first-order factor by covariate interaction term as part of the analysis; this was included for all tests of secondary hypotheses in order to: (a) reduce error variation; (b) increase sensitivity of the tests; and (c) increase the likelihood of detecting genuine differences between groups. Linearity of dependence relationships for

ANCOVAs and subsequent testing of main effects regression terms was established by evaluating plots of residuals. The groups were slightly unbalanced (29 nonsmokers and 27 smokers for tests of regional adiposity; 27 nonsmokers and 25 smokers for tests of androgen/estrogen balance and main effects interaction term testing).

The primary null hypothesis of no interactive effect of smoking with sex hormone balance and regional adiposity between the smoking and nonsmoking groups was tested by assessing homogeneity of slopes (testing for common slopes). This essentially involved calculating *separate* regression lines for regressors (independent effects) versus indicators of regional adiposity (dependent) for each group (factor level). A factorial analysis of variance (ANOVA) model was constructed which allowed testing of smoking status (a nominal factor) and a hormonal parameter (a continuous factor) *plus* an interaction term containing the respective two main effects against an indicator of adipose tissue distribution. The formal significance of the test of common slopes was assessed by looking at the factor by regressor interaction in an ANOVA table. Proportional sum of overall skinfolds ($\propto SSF_0$) was included as a regressor in the model to decrease error variation (analogous to being taken as a covariate in the ANCOVA tests of secondary hypotheses). To further decrease error variation and increase sensitivity, an interaction term containing $\propto SSF_0$ and the appropriate hormonal parameter was also included in the model, since adipose tissue interacts with hormone levels and production rates (Azziz, 1989), and these effects vary with the distribution of adipose tissue (Kirschner et al., 1990). Fatty tissue essentially both sequesters (Feher & Bodrogi, 1982) and produces (Siiteri & MacDonald, 1973) hormones, which is why it was desirable to control for the effect of this interaction on the dependent variable (an indicator of regional adiposity). Other interactions previously explored in tests of secondary hypotheses were considered redundant and omitted from the model to conserve important degrees of freedom. Only first-order interactions were considered. Type III sums of squares were calculated to remove all other effects in the model (such as unequal number of observations for each combination of factors). Least squares (adjusted) means were calculated, and dependent group means were contrasted at mean covariate (regressor) values.

CHAPTER 4: RESULTS

Sample Characteristics

Descriptive characteristics of the study sample are presented in Table 4-1. There were no significant differences between groups for age, weight, height, unadjusted or proportional sum of overall skinfolds ($\propto SSF_0$), percent body fat, hip (bi-iliac) breadth, age at menarche, or parity (parous/nulliparous). For alcohol consumption, there were no significant differences in "never" use, less than two, or two-to-six one ounce drinks (or equivalent) per week. However, there were significant differences ($p<.005$) between the groups for greater than six drinks per week: eight out of twenty-seven smokers (29.6%) reported consuming more than six ounces of alcohol per week, while zero nonsmokers consumed as much.

TABLE 4-1. Clinical Characteristics of the Study Population.

	Non smokers (n = 29)		Smokers (n = 27)		P value
	Continuous Variables				**(*t* test--two-tailed)**
	Mean	**SD**	**Mean**	**SD**	
Age (decimal years)	28.73	5.10	29.81	3.64	n.s.
Weight (kg)	58.60	7.2	59.65	9.3	n.s.
Height (cm)	164.0	6.2	165.9	6.1	n.s.
Sum of Skinfolds (mm)	111.1	40.5	117.1	49.7	n.s.
Proportional SSF_o (mm)	107.2	40.4	114.0	48.0	n.s.
Percent Body Fat[1]	19.0	7.8	20.2	7.5	n.s.
Hip Breadth (cm)	27.7	1.6	28.2	1.8	n.s.
Age at Menarche (years)	13.07	1.25	13.33	1.46	n.s.
	Categorical Variables				**(χ^2 test)**
	n (29)	*%*	**n (27)**	*%*	
Children	11	37.9	9	33.3	n.s.
never drinks alcohol	6	20.7	2	7.4	n.s.
< 2 oz. alcohol per week	12	41.4	7	25.9	n.s.
2-6 oz. alcohol per week	11	37.9	10	37.0	n.s.
> 6 oz. alcohol per week	0	0	8	29.6	<.005

[1]determined by dual x-ray absorptiometry (Lunar DPX)

Secondary Hypotheses

Adipose Tissue Distribution

A significant correlation of WHR with $\propto SSF_0$ (using $\log_{10}$ transformed values) ($r = .46$, d.f. = 54, $p<.001$) was observed for the sample group as a whole (Figure 4-1). Regression analysis indicated that over 21% of the variation in fat distribution for both groups was explained by variation in overall adiposity, even though there were no significant differences between groups for $\propto SSF_0$. Analysis of main effects variation in indicators of regional adiposity by two-way ANCOVA, taking $\propto SSF_0$ as the covariate, showed furthermore that highly significant portions of the variance in adipose tissue distribution were explained by $\propto SSF_0$ (Table 4-2). Most smoking by $\propto SSF_0$ interaction terms were also significant. The high probability values for the effect of $\propto SSF_0$ and the smoking by $\propto SSF_0$ interaction indicate that both factors were clearly useful in predicting indicators of regional adiposity, and that they therefore served a purpose in the analysis of between groups differences in adipose tissue distribution by allowing clearer assessment of the primary effect under study -- smoking status.

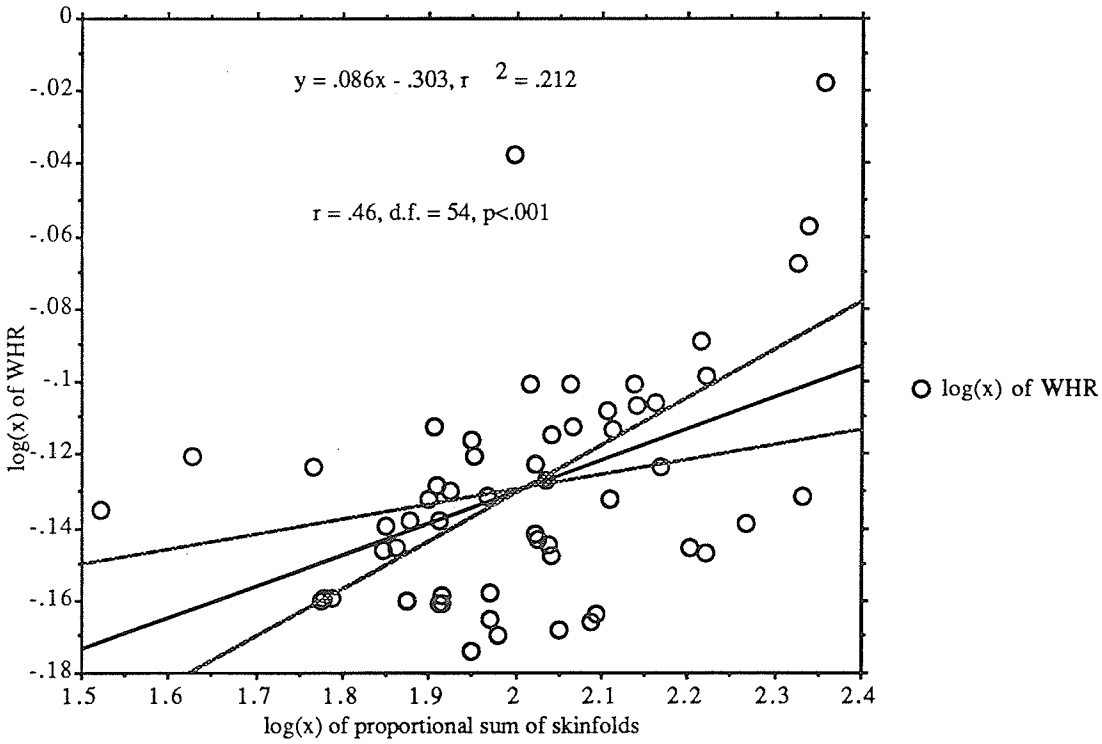

FIGURE 4-1. Correlation of log (x) of WHR with log (x) of Proportional Sum of Overall Skinfolds ($\propto SSF_0$). (Showing 95% confidence limits for slope of regression line).

TABLE 4-2. Variation in Indicators of Regional Adiposity.[1]

Variable	Source of Variation	d.f.	Sum of Squares	Mean Squares	F-test	P-value
WHR	smoking status	1	.004	.004	7.08	.0103
	proportional SSF_o	1	.011	.011	18.75	.0001
	smoking by $\propto SSF_o$	1	.005	.005	9.04	.0041
	Error	52	.030	.001		
UHR	smoking status	1	.003	.003	3.97	.0515
	proportional SSF_o	1	.021	.021	28.67	.0001
	smoking by $\propto SSF_o$	1	.004	.004	4.95	.0304
	Error	52	.037	.001		
WTR	smoking status	1	.003	.003	5.88	.0188
	proportional SSF_o	1	.007	.007	14.73	.0003
	smoking by $\propto SSF_o$	1	.004	.004	7.96	.0068
	Error	52	.026	.0005		
WHbR	smoking status	1	.012	.012	18.39	.0001
	proportional SSF_o	1	.023	.023	37.02	.0001
	smoking by $\propto SSF_o$	1	.013	.013	20.01	.0001
	Error	52	.033	.001		
waist g.[2]	smoking status	1	.010	.010	15.79	.0002
	proportional SSF_o	1	.067	.067	101.76	.0001
	smoking by $\propto SSF_o$	1	.012	.012	17.88	.0001
	Error	52	.034	.001		
umbil.g.	smoking status	1	.008	.008	8.98	.0042
	proportional SSF_o	1	.089	.089	95.85	.0001
	smoking by $\propto SSF_o$	1	.009	.009	9.87	.0028
	Error	52	.048	.001		
hip g.	smoking status	1	.001	.001	2.46	.1232
	proportional SSF_o	1	.024	.024	41.08	.0001
	smoking by $\propto SSF_o$	1	.001	.001	2.22	.1420
	Error	52	.030	.001		
thigh g.	smoking status	1	.002	.002	4.39	.0409
	proportional SSF_o	1	.030	.030	57.70	.0001
	smoking by $\propto SSF_o$	1	.002	.002	3.97	.0516
	Error	52	.027	.001		
$\propto SSF_c$[3]	smoking status	1	.003	.003	.774	.3832
	proportional SSF_o	1	2.124	2.124	504.95	.0001
	smoking by $\propto SSF_o$	1	.003	.003	.732	.3963
	Error	52	.219	.219		

[1]analyses based on $\log_{10}$ transformed values; [2]g=girth (cm); [3]$\propto SSF_c$=proportional sum central skinfolds (mm)

Table 4-3 summarizes the contribution of smoking to various indices of regional adiposity. The smoking group was characterized by significantly greater WHR ($p<.05$), WTR ($p<.05$) and WHbR ($p<.0005$). There was no significant difference in UHR ($p=.052$). For girth measurements, smokers had significantly greater waist girth ($p<.0005$) and umbilical girth ($p<.005$); upper thigh girth was significantly less in smokers ($p<.05$). There was no significant difference between groups for hip (gluteal) girth ($p=.12$), nor in proportional sum of central skinfold thicknesses ($\propto SSF_c$) ($p=.38$).

TABLE 4-3. Anthropometric Variables by Smoking Status.[1, 2]

Variable	Nonsmokers (n = 29)	Smokers (n = 27)	
WHR	.721 ± .006	.771 ± .013	†
UHR	.791 ± .008	.835 ± .016	
WTR	1.24 ± .010	1.33 ± .020	†
WHbR	2.48 ± .027	2.58 ± .052	§
waist girth (cm)	68.6 ± .908	73.0 ± 1.99	‡
umbilical girth (cm)	75.3 ± 1.17	79.2 ± 2.40	§
hip girth (cm)	95.1 ± .948	94.6 ± 1.57	
thigh girth (cm)	55.2 ± .698	54.6 ± .883	†
$\propto SSF_c$ (mm)	38.5 ± 3.17	41.8 ± 4.57	

[1]mean ± SE; [2]based on $\log_{10}$ transformed values by ANCOVA; †p<.05, ‡p<.005, §p<.0005

Sex Hormone Profiles

A significant correlation of $\propto SSF_o$ with testosterone was found for the entire sample group (r = .28, d.f. = 50, p<.05) (Figure 4-2); correlations with estradiol and indicators of sex hormone balance were not significant. Regardless, to a certain extent, androgen/estrogen balance did vary with overall adiposity, although regression analysis indicated that only 7.8% of the variation in testosterone levels could be attributed to overall

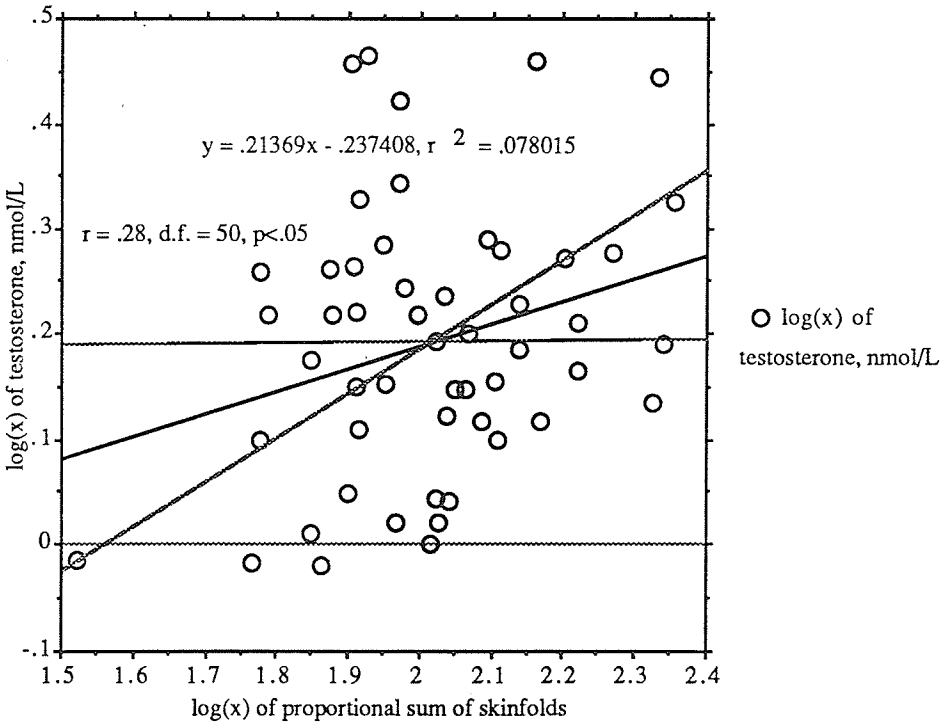

FIGURE 4-2. Correlation of log (x) of Testosterone with log (x) of Proportional Sum of Overall Skinfolds ($\propto SSF_o$). (Showing 95% confidence limits for slope of regression line).

fatness. Formal testing of main effects by ANCOVA did not show $\propto SSF_o$, as an isolated main effect, to contribute significantly to variation in hormone levels and balance, and the only significant smoking by $\propto SSF_o$ interaction observed was with SHBG ($p<.010$) (Table 4-4). The contribution of smoking to variation in sex hormone levels and balance is summarized in Table 4-5; there were no significant differences between groups -- except for SHBG -- which was significantly *greater* in smokers ($p<.010$).

TABLE 4-4. Variation in Sex Hormone Levels and Balance[1].

Variable	Source of Variation	d.f.	Sum of Squares	Mean Squares	F-test	P-value
T	smoking status	1	.031	.031	2.05	.1590
	proportional SSF_o	1	.052	.052	3.44	.0698
	smoking by $\propto SSF_o$	1	.033	.033	2.22	.1427
	Error	48	.724	.015		
E_2	smoking status	1	.050	.050	1.64	.2059
	proportional SSF_o	1	.014	.014	.46	.5002
	smoking by $\propto SSF_o$	1	.063	.063	2.07	.1564
	Error	48	1.460	.030		
T/E_2	smoking status	1	.002	.002	.07	.7999
	proportional SSF_o	1	.012	.012	.34	.5638
	smoking by $\propto SSF_o$	1	.005	.005	.13	.7188
	Error	48	1.696	.035		
SHBG	smoking status	1	.210	.210	8.402	.0056
	proportional SSF_o	1	.00023	.00023	.009	.9245
	smoking by $\propto SSF_o$	1	.202	.202	8.07	.0066
	Error	48	1.202	.025		
T/SHBG	smoking status	1	.080	.080	2.47	.1227
	proportional SSF_o	1	.059	.059	1.82	.1840
	smoking by $\propto SSF_o$	1	.071	.071	2.19	.1456
	Error	48	1.557	.032		
E/SHBG	smoking status	1	.055	.055	1.72	.1957
	proportional SSF_o	1	.018	.018	.56	.4597
	smoking by $\propto SSF_o$	1	.039	.039	1.23	.2738
	Error	48	1.54	.032		

[1]analyses based on $\log_{10}$ transformed values

TABLE 4-5. Sex Hormone Levels and Balance by Smoking Status.[1, 2]

Variable	Nonsmokers (n = 27)	Smokers (n = 25)	
testosterone, nmol/L	1.661 ± .099	1.595 ± .100	
estradiol, pmol/L	335.7 ± 26.9	266.7 ± 18.2	
T/E2 ratio[3]	5.717 ± .522	6.561 ± .515	
SHBG, nmol/L	60.79 ± 4.06	67.32 ± 5.26	†
T/SHBG	0.031 ± .004	0.027 ± .002	
E2/SHBG[3]	0.006 ± .0005	0.004 ± .0003	

[1]mean ± SE; [2]based on $\log_{10}$ transformed values by ANCOVA; [3]estradiol converted to nmol/L; †$p<.010$

Primary Hypothesis

All possible combinations of smoking by sex hormone interaction terms, and their respective main effects terms, were examined in relation to indicators of regional adiposity (dependent) utilizing the previously described factorial model which controlled for overall fatness and any fatness by sex hormone interaction. Only testosterone levels interacted significantly ($p<.05$) with smoking status; this effect was observed on both WHR and WTR. There were no significant interactions of smoking with estradiol, T/E_2 ratio, SHBG, T/SHBG, or E_2/SHBG for any girth or girth ratio. Table 4-6 presents analysis of variance tables for smoking by testosterone interactions with WHR and WTR; the interaction terms are denoted with an asterisk. Scatterplots illustrating the interactive relationship of smoking with testosterone levels, WHR and WTR are provided in Figures 4-3 and 4-4, respectively, which basically display the same relationship of smoking status with testosterone levels and regional adiposity. For nonsmokers there is essentially no change in WHR or WTR with increasing levels of serum testosterone but, in smokers, as testosterone levels increase, so do WHR and WTR. The relative impact of testosterone levels upon adipose tissue distribution clearly differs according to smoking status, and this interactive effect of smoking is significant ($p<.05$) (Table 4-6). Testosterone levels, as a main effect (independent of interaction with smoking), were also found to contribute significantly to WHR in this model ($p<.05$), as did the $\propto SSF_o$ by testosterone interaction ($p<.05$). Regarding other hormonal main effects contributions for anthropometric

TABLE 4-6. Interactive Analysis of Variation in Regional Adiposity for Smoking and Serum Testosterone Concentration (nmol/L)[1]

Dependent	Source of Variation	d.f.	Sum of Squares	Mean Square	F-test	P-value
WHR	smoking status	1	.000026	.000026	.042	.838
	testosterone (T)	1	.003	.003	4.90	.032
	*smoking by T	1	.003	.003	5.00	.030
	proportional SSF_o	1	.001	.001	.872	.355
	$\propto SSF_o$ by T	1	.003	.003	4.85	.033
	error	46	.028	.001		
WTR	smoking status	1	.000083	.000083	.156	.6943
	testosterone (T)	1	.001	.001	1.790	.1875
	*smoking by T	1	.002	.002	4.264	.0446
	proportional SSF_o	1	.001	.001	2.037	.1602
	$\propto SSF_o$ by T	1	.001	.001	1.774	.1895
	error	46	.024	.001		

[1]based on $\log_{10}$ transformed values for all variables

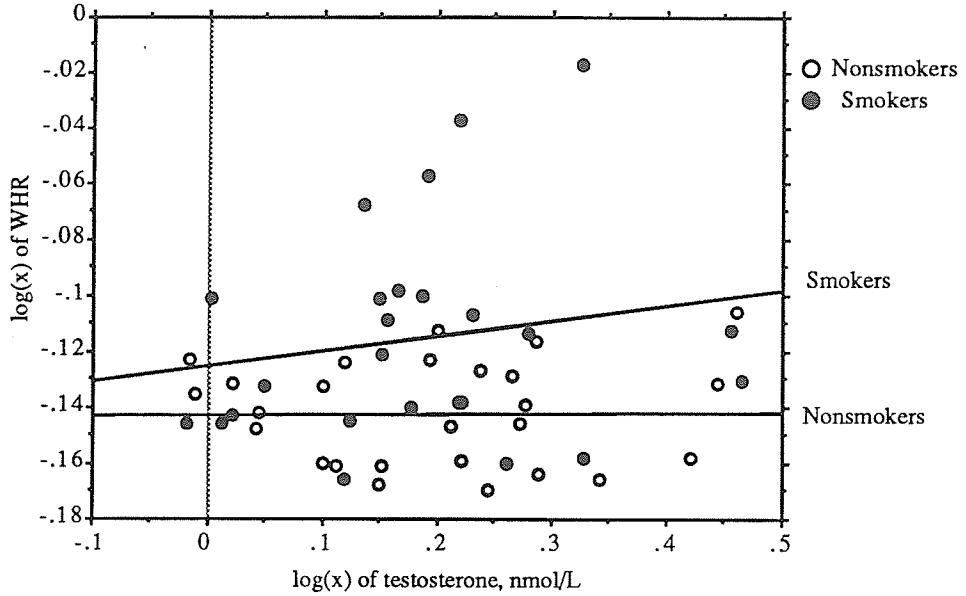

FIGURE 4-3. Interaction of Smoking with log (x) of Serum Testosterone Concentration and log (x) of WHR. (Unadjusted for $\propto SSF_o$ or $\propto SSF_o$ by testosterone interaction).

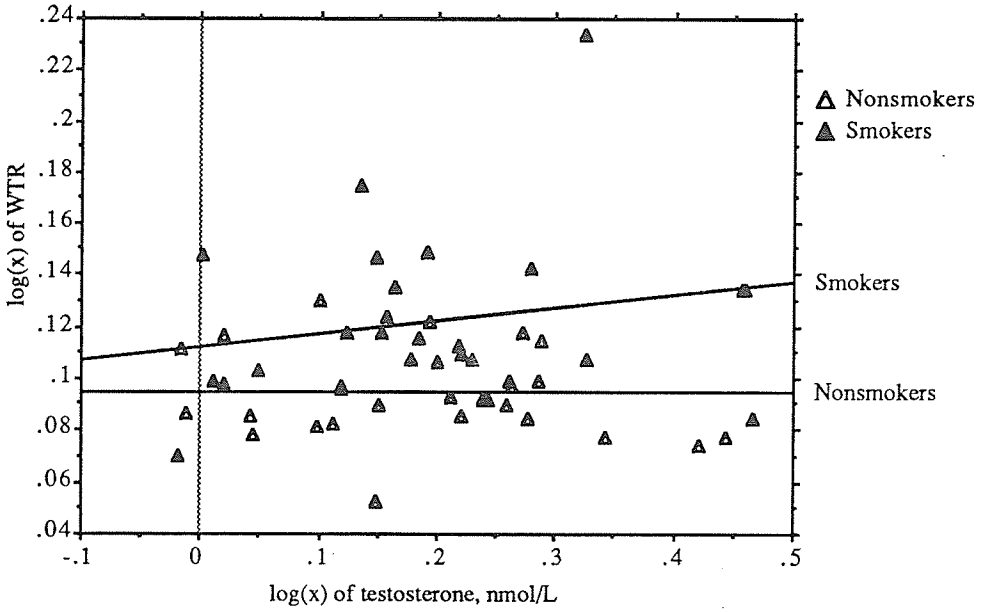

FIGURE 4-4. Interaction of Smoking with log (x) of Serum Testosterone Concentration and log (x) of WTR. (Unadjusted for $\propto SSF_o$ or $\propto SSF_o$ by testosterone interaction).

variables which did not interact with smoking, and utilizing exactly the same model (ANOVA tables not shown): (a) testosterone levels contributed significantly to WHbR (F = 4.42 with 1, 46 df; p<.05) and waist girth (F = 5.57 with 1, 46 df; p<.05); (b) the T/E_2 ratio contributed significantly to WHR (F = 5.44 with 1,46 df; p<.05), UHR (F = 7.59 with 1, 46 df; p<.010), waist girth (F = 4.78 with 1, 46 df; p<.05), umbilical girth (F =

5.80 with 1, 46 df; $p<.05$) and $\propto SSF_c$ ($F = 5.93$ with 1, 46 df; $p<.05$); and (c) the E_2/SHBG ratio contributed significantly to waist girth ($F = 5.43$ with 1, 46 df; $p<.05$). The regressing control terms included in the ANOVA model ($\propto SSF_c$ and $\propto SSF_c$ by sex hormone level or balance) were, for most dependent variables (indicators of regional adiposity), significant at at least the minimum .05 level of probability; this confirms the usefulness of their inclusion in the model with respect to accounting for more error variation and thus increasing sensitivity of analysis (results not shown, other than for testosterone, in Table 4-6). For all the aforementioned hormone and fat distribution main effects relationships, examination of scatterplots (not shown) with the best least squares line fitted, showed all slopes to be positive (independent: hormone levels or balance, dependent: adipose tissue distribution).

CHAPTER 5: DISCUSSION

The primary null hypothesis of no interactive effect of smoking with sex hormone levels and regional adiposity in smoking and nonsmoking premenopausal females was rejected on the basis of a significant interaction between smoking status, serum testosterone concentration and WHR and WTR. The secondary null hypothesis of no difference in adipose tissue distribution between premenopausal female smokers and nonsmokers was rejected on the basis of greater WHR, WTR and WHbR in smokers. The secondary null hypothesis of no difference in sex hormone levels or balance between premenopausal female smokers and nonsmokers was rejected, in principle, on the basis of significantly greater serum concentrations of SHBG in smokers relative to nonsmokers.

Sex Hormones and SHBG

Acceptance of the hypothesis that serum levels of SHBG differ between smokers and nonsmokers must be viewed with caution in that one cannot inductively transpose this finding as evidence of a similar directional difference in androgen/estrogen balance. SHBG is generally considered to be an indicator of androgen/estrogen balance, high serum levels representing estrogenic dominance and low serum levels representing androgenic dominance (Vermeulen et al., 1969; Enriori & Reforzo-Membrives, 1984; Peiris et al., 1989), and this author was no different from others in making this empirically-based assumption. But in smokers, the nature of the observed differences in serum levels of testosterone and estradiol, while not statistically significant, were in the opposite direction from that which might be inferred from high levels of SHBG in nonsmokers. While serum testosterone levels were about 4% lower in smokers, estradiol was lower by almost 21% and the T/E_2 ratio was greater by almost 13%. These differences suggest that in female smokers, estradiol levels are substantially decreased relative to a negligible decrease in testosterone levels (that is, androgen/estrogen balance is shifted in favor of androgens); they also suggest that the statistical power of the study was too low to confirm the biological significance of these differences. Discrepancies between the nature of observed differences in testosterone and estradiol levels and the T/E_2 ratio -- albeit statistically insignificant -- and the nature of the observed difference in SHBG -- which was statistically significant -- suggest that smoking has differential effects on the levels of SHBG and sex steroids. If this is the case, then: (a) SHBG cannot be considered a valid marker of relative

androgenic/estrogenic balance when smoking is a factor; and (b) rejection of the null hypothesis that smoking does not alter androgen/estrogen balance, on the basis of greater serum concentrations of SHBG in smokers, is inappropriate. What is theoretically relevant are the directions of the differences in testosterone and estradiol levels, even though statistically nonsignificant. It is suggested that greater statistical power be considered in future studies attempting to assess potentially subtle differences in endogenous reproductive hormone levels during the normal menstrual cycle.

Insofar as rejection of the pertaining null hypothesis is unjustified, the finding of greater concentrations of SHBG in female smokers is important and most relevant. This observation confirms that of the only other study to consider SHBG levels between premenopausal smokers and nonsmokers (Moore et al., 1987), which found serum concentrations of SHBG to be 13% higher in smokers; statistical significance was barely missed (p=.051). As discussed, greater levels of SHBG in female smokers imply greater serum levels of estrogens and/or lower serum levels of androgens, but such findings have never been made. Rather, the *converse* has been observed, although some studies have failed to observe any differences between smokers and nonsmokers. For example, it has been shown that female smokers display significantly elevated serum concentrations of androstenedione, DHEA-S and testosterone in association with serum estradiol and estrone levels not significantly different from those of nonsmokers (Friedman et al., 1987; Khaw et al., 1988), while pregnant smokers display lower serum estrogens than pregnant nonsmokers (Mochizuki et al., 1984; Bernstein et al., 1989). Other studies, observing elevated serum androstenedione, have found no differences in DHEA-S or testosterone (Cauley et al., 1989; Longcope & Johnston, 1988). Notwithstanding the possibility that female smokers truly are characterized by greater degrees of androgenic relative to estrogenic activity than nonsmokers, even evidence of no such difference -- in the face of elevated plasma SHBG in smokers -- supports the contention that factors other than reproductive hormones regulate the concentration of SHBG in those women who choose to smoke.

There are other situations in which SHBG also appears invalid as an androgen/estrogen marker. Obese males display low concentrations of SHBG in association with elevated levels of estrogens and low levels of androgens (Zumoff, 1988; Barbato & Landau, 1984), and obese females display low levels of SHBG concomitantly with elevated levels of estrogens and androgens (Davidson et al., 1981; Zumoff, 1988). Anorexia nervosa, known to be associated with a hypoestrogenic state, is conversely associated with greater levels and greater binding capacity of SHBG (Pugeat et al., 1988a). Rapid decreases in binding capacity and levels of SHBG occur with return to normal

weight (Pugeat et al., 1988a). Osteoporosis is also associated with a hypoestrogenic state (Friedman et al., 1987); however, bone density is inversely correlated with both SHBG binding capacity (Pugeat et al., 1988a) and serum concentration of SHBG (van Hemert et al., 1989).

Thus, while in most situations SHBG provides a reasonable indication of relative androgenic/estrogenic activity, the above-noted phenomena illustrate that other, unknown factors may regulate the concentration or binding capacity of SHBG. These could be both hormonal (non-reproductive) and/or non-endocrine; there is accumulating evidence for a relationship between SHBG levels, energy balance, diet, physical activity and lipid metabolism (Pugeat et al., 1988a). Regulation of the metabolic clearance rate and the influence of non-endocrine factors on SHBG production are still a matter of controversy. In smokers, greater levels of SHBG could possibly be due to greater degrees of thyroid activity: thyroid hormones are known to increase SHBG levels in normal subjects (Anderson, 1974), and smoking alters thyroid function (Gofin et al., 1982; Sepkovic et al., 1984). In view of the direction of the observed differences in testosterone and estradiol in the present study, it is stressed that the finding of greater serum SHBG levels in female smokers in no way implies increased estrogenic relative to androgenic function in smokers; this finding likely represents other extraneous factors which were neither anticipated nor controlled.

Regional Adiposity

The observation that premenopausal smokers are characterized by a greater degree of android adiposity than nonsmokers is consistent with other observations of pre- and postmenopausal women (Tonkelaar et al., 1989; Kaye et al., 1990) as well as observations of other groups not stratified for gender or menopausal status (Haffner et al., 1986a; Barrett-Connor & Khaw, 1989; Cox, 1989). All these other studies utilized the WHR to distinguish abdominal from gluteal-femoral adiposity and most, but not all, adjusted WHR for overall fatness (BMI). This study did not adjust indices of regional adiposity for BMI; a proportional sum of eight skinfolds was considered to be a superior covariate ($\propto SSF_o$). Irrespective of methodological differences, the intention was the same: to remove the confounding influence of overall fatness on adipose tissue distribution. In view of this, it is interesting to understand the effect of such an adjustment on indices of regional adiposity when there are subtle but statistically insignificant differences in overall adiposity between sub-groups within a sample.

Although not reported in Chapter 4, the present study assessed the significance of between groups differences in regional adiposity *unadjusted* for overall fatness using a simple two-group ANOVA which enables comparisons with the reported ANCOVA results. There is no intention of reporting the ANOVA results here, but some theoretical concepts deserve discussion in that they have methodological implications. The statistical significance of between groups differences in WHR, UHR and WTR was found to *decrease* after adjustment for $\propto SSF_o$. The only other study specifically concerning premenopausal smokers and nonsmokers (Tonkelaar et al., 1989) found that adjustment for overall fatness (BMI) *increased* the significance of between groups differences in WHR. That significance increased in this latter study implies an inverse relationship between overall fatness and WHR, something to be expected when considering the inverse relationship of smoking to body weight and fatness in light of the positive relationship of smoking with WHR. Indeed, smokers in this latter study had slightly (but not significantly) lower BMIs than the nonsmokers. Smokers in the present study were slightly (but not significantly) fatter than the nonsmokers (greater $\propto SSF_o$), which likely explains why the significance of differences in WHR, UHR and WTR decreased. In view of the opposite nature of adjusted results in each respective study, it is suggested that studies contrasting any indirect index of regional adiposity always adjust for overall fatness -- even when there are no significant differences in mean fatness levels -- in view of the tendency for fat distribution to vary with overall fatness. Failure to do so could result in the creation of "additional" differences in whatever dependent variable was being analyzed, and these differences would have nothing to do with the effect under study. Valid comparisons are only enabled when confounding influences are controlled.

The present study also considered differences in WHbR, waist, umbilical, hip and thigh girths between smokers and nonsmokers. There were no significant differences between groups for any of these variables until adjustment for $\propto SSF_o$, at which point all became significant except hip girth. Of particular interest were remarkable changes in the significance of between groups differences in WHbR (from p=.091 to p=.0001) and waist girth (from p=.052 to p=.0002). These results, when considered with the lack of effect of smoking on hip girth, suggest that the region primarily affected by smoking is the waist, and that this effect is independent of overall adiposity. Hence the greater P-value of WHbR (p=.0001) relative to that of WHR (p=.010) after each was adjusted for overall fatness. It should be stressed that the high statistical significance of greater WHbR in smokers (relative to the statistical significance of WHR) is of biological significance (in terms of a primary effect of smoking on the waist) only in view of the observed lack of significant differences in hip girth between groups. If this were not the case, it is conceivable that

greater statistical significance of WHbR relative to WHR could merely reflect the fact that hip girth varies more than hip breadth (waist girth remaining constant for each index), placing limitations on the amount of variation which the WHR could account for when the waist is the region affected most by smoking. In men, there is a significant and positive association of WHbR with smoking (Troisi et al., 1990), and the effect of smoking in the promotion of android adiposity also appears primarily mediated by effects on waist circumference (Selby et al., 1990). Furthermore, a lack of effect of smoking on hip girth has also been reported in men (Shimokata et al., 1989; Selby et al., 1990).

The remaining two circumference measures considered in the present study were umbilical and thigh girths. While adjusted umbilical girth was significantly greater in smokers, this effect was of much less magnitude than the effect of adjustment for overall fatness on waist girth; this, and the lack of statistically significant differences in UHR further support an anatomically distinct effect of smoking on the waist region -- as distinguished from the umbilical region. Observations of significant differences between groups for adjusted thigh girth and WTR (Table 4-3) indicate an additional effect of smoking on femoral adiposity, one which is perhaps secondary to effects on the waist.

Not yet discussed is the central sum of skinfold thicknesses ($\propto SSF_c$) contrasted between smokers and nonsmokers. That there were no significant differences between groups for this measure, has important connotations in view of significant differences in girths and girth ratios which suggest a primary effect of smoking at the waist, or about the abdominal region in general. Clearly, $\propto SSF_c$ measures something quite different from what girths and girth ratios assess. Subcutaneous central adipose tissue is represented by the $\propto SSF_c$; intra-abdominal and overlaying adiposity is represented by girths and girth ratios. Consequently, the lack of significant between groups differences in $\propto SSF_c$ suggests that effects of smoking are not just mediated centrally, but that they are mediated internally; that is, smoking appears to promote intra-abdominal adiposity. Confirmation of this hypothesis would require assessment of intra-abdominal adiposity in smokers versus nonsmokers by an *in vivo* method such as computed tomography (CT) or nuclear magnetic resonance imaging (NMRI).

The WHR and WHbR appear to be the most sensitive and useful of the indirect indices applied to assess differences in regional adiposity between smokers and nonsmokers. The WTR was also useful, in that it enabled assessment of possible effects of smoking on the femoral fat depot, but the UHR was relatively insensitive. Results of girth measurements are consistent with and support the above. The $\propto SSF_c$ was not useful in assessing effects of smoking, other than as a means of inferring -- in conjunction with girths and girth ratios -- whether effects on adiposity are internal or subcutaneous. The

author is not aware of any other studies which have considered the utility of different indices of adipose tissue distribution when studying effects of smoking.

Interactive Effects of Smoking, Sex Hormones and Regional Adiposity: Implications and Speculations

The finding of a significant interaction of smoking with serum testosterone concentration and android adiposity in premenopausal women is of considerable biological importance. This original observation provides some explanation for recently noted relationships of abdominally localized fat with cigarette smoking in humans. Based on this interaction, it is a reasonable hypothesis that by effecting a shift in the degree of androgenic relative to estrogenic activity, smoking induces differential redistribution of body fat in favour of the abdominal depot in reproductively capable women whose primary fat storage depot is normally the gluteal-femoral region. However, only longitudinal observation can resolve this hypothesis, a rather difficult undertaking from an ethical standpoint in that smoking cannot be utilized as an active intervention. One way around such a dilemma might be to examine dose-response relationships of cigarette smoking with regional adiposity and sex steroid levels in relatively lengthy prospective studies. Such investigations would have the additional benefit of being able to identify voluntary interventions in the form of initiation or cessation of cigarette smoking, perhaps being able to separate out enough of these subjects for independent study.

There are several apparent and implied consequences of smoking-induced promotion of android adiposity in premenopausal women. The first concerns increased health risk due to abdominally localized body fat, a relationship which is independent of overall adiposity. That this appears to be induced at a time when women are normally protected from health risk by either their estrogenic dominance and/or their predominantly gynoid fat mass, is cause for concern. No clear distinction can currently be made as to whether increased health risk would be due to an altered androgen/estrogen profile or abdominally localized fat *per se*; both are likely involved (refer to Chapter 1 -- Pathogenesis of Complications Associated with Regional Adiposity). Despite the present study's observation that smokers and nonsmokers were not significantly different from each other in terms of overall fatness (Table 4-1), smokers, as a group, are generally less fat and of lower bodyweight than nonsmokers (Garn, 1985b; Kromhout et al., 1988; Comstock & Stone, 1972). With respect to Figures 1-1 and 1-2, which respectively illustrated relationships between BMI, all cause mortality and cardiovascular disease, it is plain that

there is an increase in morbidity and mortality at low BMIs, a category into which smokers generally fit. Awareness of the association of smoking with low bodyweight or fatness and high degrees of android adiposity helps to explain this apparent inconsistency in the general positive relationship of bodyweight or fatness with morbidity and mortality, at least in terms of smokers.

Another consequence of android adiposity in premenopausal smokers concerns potential changes in the disposition of various adipose tissue depots. Human adipose tissue depots are known to respond differentially according to gender and menopausal status (Vague et al., 1985). There is also differential response during pregnancy and lactation (Smith U., 1985; Rebuffé-Scrive et al., 1985a). The degree to which smoking effects changes in regional adipocyte metabolism has not yet been investigated, but it is possible that metabolic characteristics and responses of the abdominal and gluteal-femoral regions in men, postmenopausal women and premenopausal smokers might be remarkably similar in view of their similar body fat distributions. Sex hormone balance is reasonably similar in men and postmenopausal women (Samoljik et al., 1977; Eldrup et al., 1987) and, while a similarity between postmenopausal women and premenopausal smokers remains to be confirmed, preliminary evidence of differences in serum androgen and estrogen levels in smokers supports this contention (Hartz et al., 1987; Lawrence et al., 1987; Barrett-Connor & Khaw, 1987). Of further importance is the need for understanding of the effect of altered body fat distribution in *pregnant* smokers; for example, what effect would diminution of the gluteal-femoral fat depot (in favor of the abdominal) have on energy supply during pregnancy and lactation? This is an important question in view of the specific reproductively-related role the gluteal-femoral region seems to play in premenopausal women (Rebuffé-Scrive et al., 1985a). Notably, the present study found significantly greater WTR and significantly smaller thigh girth in smokers, which suggests that the femoral fat depot is a less viable storage site in smokers.

That the present study found smoking to interact significantly with serum testosterone, WHR and WTR, but no significant differences between groups for serum testosterone or estradiol, raises some interesting points and questions. First of all, the primary design of the study -- to test for interaction -- is validated, in that simply testing groups to see if androgen/estrogen levels made a contribution to body fat distribution would have been pointless, given the fact that the relative contribution of testosterone to WHR and WTR was found to vary with smoking status. That is, the relative effect of testosterone on android adiposity was greater in smokers. It therefore cannot be assumed that the basic relationship of testosterone to WHR or WTR is the same for each group (see Figures 4-3 and 4-4). The overall implication is that smoking is an important factor which

deserves attention when making assessments of regional adiposity in view of an interactive effect with testosterone. This conclusion holds even though no significant differences were found between groups for any serum hormone concentration.

The question must be raised as to whether there truly are differences in sex steroid levels between smokers and nonsmokers. In contrasting different groups, it is exceedingly difficult to obtain a representative indication of reproductive hormone concentrations during the normal menstrual cycle due to intra- and inter-individual variation in menstrual cycle timing. As already discussed, the present and other (Moore et al., 1987) findings suggest that SHBG cannot be utilized in smokers as a relatively stable indicator of sex steroid balance, one capable of reflecting tissue sensitivity and the impact of fluctuating reproductive hormone levels. Without question, although both testosterone and estradiol fluctuate with the menstrual cycle, the variability of estradiol is much greater, and it is consequently more difficult to obtain a reasonable indication of mean levels at any point. Thus it is possible that between smokers and nonsmokers, there are differences in endogenous reproductive hormone levels, and that current methodological and technological procedures are simply not capable of detecting them.

Though the only endocrine parameter found to vary between groups was serum SHBG concentration, and no significant interaction between smoking, SHBG and any indicator of regional adiposity was observed, it was desirable to explore the direction of the relationship of the groups with SHBG and regional adiposity. Figure 5-1 presents a scatterplot of WHR against serum SHBG with the best least squares line fitted for each

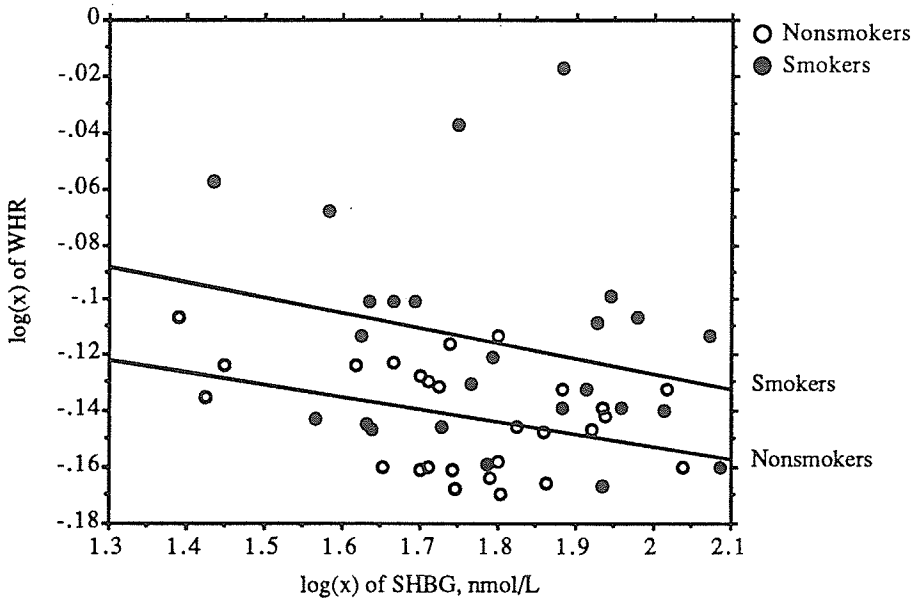

FIGURE 5-1. Scatterplot of log (x) of WHR versus log (x) of Serum SHBG Concentration for Smoking and Nonsmoking Women.

group. That the slopes for the relationship of each group are roughly parallel is consistent with the lack of interaction as tested by analysis of variance; that both are negative is consistent with other studies noting inverse relationships of serum SHBG levels with WHR (Evans et al., 1983). Thus, even though serum SHBG was significantly greater in smokers, the general inverse relationship between WHR and SHBG still holds, which is consistent with general associations of androgenic activity and high WHR, and estrogenic activity with low WHR (Evans et al., 1988). The above is a crucial observation, in that when serum SHBG is related to regional adiposity, the directional shift in serum androgen/estrogen balance implied by elevated SHBG in smokers is negated. This graphic representation is confirmed by the tested lack of significant interaction between smoking, SHBG and any indicator of regional adiposity.

As discussed, "free" (non-SHBG-bound) portions of sex steroids were estimated based on the free estrogen index (FEI; FEI = E_2/SHBG) and free androgen index (FAI; FAI = T/SHBG) (Carter et al., 1983). "Free" portions of total serum levels are thought to represent that which is biologically active and therefore may be more important than total serum levels (Siiteri, 1987; Bruning, 1987; Moore et al., 1987; Bruning et al., 1988). Yet no significant differences were found between groups for estimated levels of "free" sex steroids, nor were any significant "free" sex steroid interactions with smoking and adipose tissue distribution observed. In view of the observed significant interaction of smoking with (total) serum testosterone concentration and regional adiposity, the lack of a similar interaction with "free" testosterone forces reconsideration of the concept that "free" hormone levels are more representative of biological activity than total serum levels. This statement, of course, is entirely dependent upon the assumption that the index utilized to estimate "free" testosterone levels is valid. Hypothesizing, it is possible that smoking is associated with impaired sex steroid-binding concomitant with greatly increased SHBG levels, but only direct measurement of free steroids can confirm this.

While unadjusted differences in "free" sex hormone levels were statistically non-significant, they were consistent with directional differences in total serum steroid levels and are worthy of some comment. In smokers the FAI was 14.5% lower, and the FEI 26.6% lower, than in nonsmokers; low levels in smokers of estimated "free" sex steroids are in agreement with elevated SHBG levels. It should be noted that the substantial difference in the FEI was statistically significant at p=.014 until adjustment for overall fatness, which indicates that overall adiposity is useful in predicting "free" estradiol, a phenomenon noted by others (Azziz, 1989). Importantly, percent differences in FAI and FEI indicate, in a relative sense, much lower "free" estradiol levels than "free" testosterone levels. That both total and "free" levels of estradiol were lower in smokers (by 20.6% and

26.5%, respectively) suggests a possible synergistic effect with testosterone and regional adiposity, in that the abdominally-specific lipolytic effect of estradiol (Rebuffé-Scrive, 1988),(Rebuffé-Scrive et al., 1988b) could be inhibited, therefore indirectly promoting (or allowing) abdominal lipogenesis. But because no interactive effect of smoking with either total or "free" estradiol was statistically confirmed, the foregoing is speculative.

General Relationship of Androgen/Estrogen Balance to Regional Adiposity

Although the fundamental purpose of this study was to explore differences between premenopausal smokers and nonsmokers in terms of androgen/estrogen levels and regional adiposity, and interaction between these factors, there is still a need for further confirmation and support of the sexually dimorphic role of sex steroids in the regulation of adipose tissue distribution. Toward this end, findings of significant main effects contributions of testosterone, the testosterone/estradiol ratio and the free estradiol index to various indices of adipose tissue distribution, for the sample group as a whole, are of considerable importance.

Serum testosterone was found to contribute significantly to WHR, WHbR and waist girth. The author is not aware of any other study which has utilized a factorial analysis of variance design to investigate the relationship of sex steroids with regional adiposity, but the significant contribution of serum testosterone levels to WHR is in agreement with positive correlations of total serum testosterone to WHR in women (Kirschner et al., 1990; Hauner et al., 1987). Similar correlations have been made (in women) with free testosterone (Evans et al., 1983; Evans et al., 1988; Peiris et al., 1987a; Seidell et al., 1990), but the present study found no significant contribution of "free" testosterone -- as estimated by the free androgen index -- to variation in any anthropometric variable. This could simply represent a methodological incompatibility, in that specific assays for free testosterone were conducted in these other studies. Findings of significant contributions of serum testosterone to WHbR and waist girth are unique, and have not been reported for *any* sample population. The finding that much of the variance in waist girth is explained by testosterone is very supportive of the association of androgens with abdominal adiposity *per se*.

Significant contributions of the testosterone/estradiol (T/E_2) ratio to WHR, UHR, waist girth, umbilical girth and $\propto SSF_c$ support the concept that adipose tissue distribution is a function of the degree of androgenic relative to estrogenic activity. These observations suggest, furthermore, that in premenopausal women, android fat distribution is a

consequence of either elevated androgens and/or decreased estrogens relative to a normally dominant estrogenic profile of which gynoid fat mass is a function. It is also apparent that androgen/estrogen balance is of little consequence in determining hip girth, which allows speculation that other factors, perhaps muscular-skeletal, are more important determinants than adiposity for this girth. In attempting to assess relative androgenic/estrogenic activity in relation to regional adiposity, serum SHBG has consistently been utilized as an indicator of androgen/estrogen balance; inverse associations of WHR with SHBG are numerous (Evans et al., 1983; Lapidus et al., 1986; Hauner et al., 1988; Haffner et al., 1989; Ridder et al., 1990; Kirschner et al., 1990). However, no studies examining the relationship of the T/E_2 ratio to indices of regional adiposity have been reported. That the present study found significant contributions of the T/E_2 ratio to body fat distribution is evidence that the theoretical concept of androgen/estrogen balance has empirical utility in relation to adipose tissue distribution. Furthermore, utilization of the T/E_2 ratio in this regard needs no resort to assumptions about the nature of the behavior of SHBG as an indicator of androgen/estrogen balance, assumptions which cannot always be upheld, as this author and others (Pugeat et al., 1988a) have proposed.

The finding that "free" estradiol, as estimated by the free estradiol index (FEI), contributed significantly to waist girth is most interesting. In women, it is known that sub-normal levels of SHBG are associated with elevated free estradiol and free testosterone (Zumoff, 1988); this commonly occurs in obesity. Yet serum SHBG was normal in nonsmokers, elevated in smokers, the mean level of SHBG for the overall sample group being normal but slightly higher than average. Given that "free" estradiol was not elevated due to low SHBG, it is difficult to understand a significant contribution to waist girth. It is possible this is a spurious finding, in view of the fact that waist girth is partially dependent on overall fatness, and that a significant "free" estradiol by overall fatness interaction was concomitantly observed in testing factorial effects on waist girth.

Limitations and Suggestions

The fundamental limitation of the present study is its cross-sectional nature, which does not permit any causal inference. As discussed, longitudinal replication of these findings is necessary, and would perhaps enable understanding of the mechanism by which smoking interacts with sex steroids and regional adiposity. Such study might furthermore clarify the dichotomous nature of elevated serum SHBG and increased androgenic relative to estrogenic activity in premenopausal smokers. That this study has not confirmed other

observations of low levels of SHBG in women with android adiposity (Haffner et al., 1989; Kirschner et al., 1990) appears to be attributable to cigarette smoking, but the overall concept that SHBG levels primarily reflect androgen/estrogen balance may need to be revised. The second most serious limitation of the current study is that a single sample of blood was assumed to provide a reasonable indication of endogenous sex steroid levels. In view of the directional but non-significant differences between groups in serum testosterone and estradiol levels, a logical extension would be to contrast mean sex steroid concentrations from multiple samples of blood drawn at standardized points of the menstrual cycle over several months. Such a design would enable better assessment of any difference between smokers and nonsmokers. A minor limitation in interpreting results is the observation that eight out of twenty-seven smokers reported alcohol consumption in excess of six ounces per week; smoking and drinking were somewhat related behaviors. There were not, however, any differences between groups for zero, less than two, or two-to-six ounces of alcohol consumption per week, and the groups were judged to be comparable. Any effect of this minor difference in alcohol consumption on dependent variables is unknown.

Extensions of the current study not suggested above might include assessment of the effect of smoking on other steroid hormones -- such as progesterone and cortisol -- in relation to body fat distribution. Because progesterone is implicated in the control of femoral lipogenesis (Rebuffé-Scrive et al., 1983), an inhibitory effect of smoking on progesterone might explain observations of less femoral adiposity in premenopausal smokers in the present study. With respect to Chapter 2, Section II, cortisol is implicated in android adiposity, and smokers are characterized by elevated serum cortisol. The involvement of cortisol in adipose tissue distribution has not been adequately researched, and there has never been reported an investigation of interactive effects of smoking with cortisol and regional adiposity.

Summary and Conclusions

The purpose of this treatise was: (a) to illustrate, from the literature, that obesity *per se* is at best a crude indicator of health risk, whereas the distribution of adipose tissue is more clearly related to health risk, and that relationships between body fat distribution and health risk are *independent* of the overall adipose tissue mass; (b) from the literature, to implicate and describe the sexually dimorphic effects of reproductive hormones in terms of the distribution and disposition of adipose tissue; (c) to illustrate from the literature

independent associations of cigarette smoking with altered body fat distribution and atypical reproductive hormone serum levels, production and excretory rates; and (d) to report a study undertaken to investigate a potential interrelationship between smoking, sex hormone balance and adipose tissue distribution in young women.

Fifty-six premenopausal females ages 20-35 were selected for observation after careful screening for any factors which may have confounded interpretation of results. The sample was sub-divided into nearly equal smoking and nonsmoking groups. All subjects underwent a comprehensive battery of anthropometric measurements. Blood samples were drawn in the mid-follicular phase and serum analyses conducted for sex hormones (testosterone and estradiol) and SHBG (an indicator of sex hormone balance). All variables were adjusted for overall fatness. Smokers were characterized by significantly greater degrees of android adiposity (greater WHR, WTR and WHbR) than nonsmokers, reflecting, in terms of adipose tissue distribution, greater health risk. Non-significant differences in serum testosterone and estradiol levels implied a trend towards elevated androgenic activity in premenopausal smokers, but smokers were concomitantly characterized by significantly greater levels of SHBG. Significant interactions of smoking with serum testosterone concentrations and WHR and WTR were observed. For both the smoking and nonsmoking groups, significant contributions of testosterone, the testosterone/estradiol ratio and "free" estradiol were made to indices of adipose tissue distribution.

Within the delimitations noted, the following conclusions are justified: (a) sex steroid levels and balance make significant contributions to adipose tissue distribution; (b) smoking is associated with android adiposity independent of overall adiposity; (c) smoking may increase androgenic relative to estrogenic activity, but there are differential effects of smoking on serum sex steroid and SHBG levels; and (d) smoking interacts with serum testosterone levels and regional adiposity.

These conclusions suggest that in premenopausal women, sex hormones regulate body fat distribution, and that smoking changes adipose tissue distribution from the gynoid to android configuration through interactive effects with sex hormones.

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APPENDIX A

Estimation of Subject Number from Calculations of Statistical Power

APPENDIX A

Estimation of Subject Number from Calculations of Statistical Power

The number of subjects (N) was dependent upon (a) the number of subjects in each of the two treatment subgroups, which was in turn dependent upon (b) variation in the parameters being assessed -- regional adiposity and relative androgenic/estrogenic activity.

From Effect Size of Smoking on Relative Androgenic/Estrogenic Activity

Relative androgenic/estrogenic activity was assessed by determining serum levels of estradiol, testosterone and SHBG; the "free" (unbound) portions of these were also estimated. Each of these measures possesses its own inherent variability, and each therefore influenced the number of subjects required. Unfortunately, there was no information in the literature regarding mean plasma levels, and standard deviations from these means, for any of the above measures in premenopausal smokers versus nonsmokers. There was, however, information available regarding differences in estradiol degradation between premenopausal female smokers and nonsmokers (recall that increased hepatic metabolism of estrogen in female smokers is the most evident "antiestrogenic" effect). From these data it was possible to calculate statistical power and ß level and, consequently, estimate the magnitude of the observed Effect Size. In lieu of more definitive information, this Effect Size was postulated to be similar for plasma estradiol levels, and subject number was determined based on possible differences in estradiol levels. The study of Michnovicz (1988) on the extent of estradiol metabolisation in premenopausal smokers versus nonsmokers provided the following data from which power and ß level were calculated.

$$N = 26;\ n_{smokers} = n_{nonsmokers} = 13$$

E_2 metabolism: greater in smokers ($p<0.02$)

$$H_A - H_O = 1.9;\ \sigma = 0.5$$

Calculation of ß level and Power:

Since $p<0.02$, then $z_\alpha = 2.33$ (two-tailed). $z_\beta = ?$

According to Hassard (1990), for two sample means:

$$z_\alpha + z_\beta = \text{Power Index (PI)} = \left(\frac{\sqrt{n}}{\sqrt{2}}\right)\left(\frac{(H_A - H_O)}{\sigma}\right)$$

$$\text{and } n = 2\left[PI\left(\frac{\sigma}{(H_A - H_O)}\right)\right]^2,$$

where n is the number of subjects required for each of two subgroups whose means are being contrasted.

$$PI = \left(\frac{\sqrt{13}}{\sqrt{2}}\right)\left(\frac{1.9}{0.5}\right) = 9.69$$

$$z_\beta = PI - z_\alpha = 9.69 - 2.33 = 7.36$$

Power therefore approaches 1.00, and ß approaches 0.00.

Because (a) ß is so low and Power is so high, and (b) sample size is relatively small, a large Effect Size is indicated. In lieu of knowing $[\sigma/(H_A - H_O)]$, Cohen (1977) suggests, for a large Effect Size, replacing $[\sigma/(H_A - H_O)]$ with (1/0.80). Levels of α and ß were therefore defined so that subject number could be calculated.

Allowable risk of Type I error was 5%; thus, $\alpha = 0.05$. Power was set at 0.80; therefore, ß = 0.20 and risk of Type II error was 20%. The rationale for setting ß at this low, but still acceptable, level was that a higher level would have resulted in an impractically large sample.

Calculating required number of subjects for each of the two groups:

$$\alpha = 0.05,\ z_\alpha = 1.64 \text{ (one-tailed)}$$

$$ß = 0.20,\ z_ß = 0.84 \text{ (one-tailed)}$$

$$n = 2\left[(1.64 + 0.84)\left(\frac{1}{0.80}\right)\right]^2 = 19.22 \approx 20$$

Twenty subjects per group were required based on the estimated Effect Size of smoking on sex hormone levels.

From Relative Impact of Smoking on Regional Adiposity

Regional adiposity was assessed by use of the WHR. The previously-mentioned study of Tonkelaar (1989) which specifically contrasted WHR of premenopausal female smokers with nonsmokers was directly applicable and was utilized to calculate subject number in this case.

$$H_A - H_O = 0.02;\ \sigma = 0.028$$

Using levels of α and ß as set above, required number of subjects for each of the two subgroups was:

$$n = 2\left[(1.64 + 0.84)\left(\frac{0.028}{0.02}\right)\right]^2 = 24.11 \approx 25$$

Twenty-five subjects per group were required based on the Relative Impact of smoking on WHR.

Since fewer subjects were required to observe an appropriate effect of smoking on sex hormone levels than those required to observe an appropriate effect on WHR, total number of subjects was based on this latter calculation.

For each group, a minimum of 25 subjects was required; the minimum number of total subjects required was 50.

APPENDIX B

Information to Participant and Informed Consent Form

APPENDIX B

Information to Participant and Informed Consent Form

Sport & Exercise Sciences Research Institute
University of Manitoba

Smoking, Adipose Tissue and Bone Mineral Density Study:
Information to Participant and Informed Consent Form

Investigator: M. Daniel, B.Sc., Graduate Student
Supervisor: A. D. Martin, Ph.D., Director,
Sport & Exercise Sciences Research Institute,
University of Manitoba

Background:

Overweight, or obesity, has long been associated with health complications. However, in recent years, information has come to light indicating that, except in extreme obesity, it is not so much the *amount* of fat which is indicative of health risk, it is the *location* of the fat. The distribution of fat is therefore of equal, if not more, importance than the amount of fat.

At the same time, it has become apparent that there are age- and sex-dependent differences in body fat distribution. Women, prior to menopause, predominantly localize fat on or about the hips and thighs; this is known as *gynoid* fat distribution. Men and post-menopausal women predominantly localize fat about the abdomen; this is known as *android* fat distribution. It presently appears that the relative activity of the female sex hormones (estrogens) strongly influences gynoid body fat distribution, and that the relative activity of the male sex hormones (androgens) influences android body fat distribution.

Smoking, an independent variable, appears to influence sex hormone levels, body fat distribution and the composition of lean tissues -- such as bone. These effects appear to be completely independent of a well-noted characteristic of smoking: smokers, as a group, weigh less and are less fat than their nonsmoking peers. Specifically, in pre-menopausal females, smoking is associated with: (a) lower estrogen levels and/or greater androgen levels; (b) greater android (abdominal) body fat; and (c) lower bone density. However, these three effects have never been observed or studied in the same group of women, something which would imply that by altering the balance of sex hormones, smoking increases android body fat and decreases bone density.

The hypothesis of this study, then, is that a group of pre-menopausal female smokers contrasted against a group of nonsmoking pre-menopausal female smokers will show (a) greater androgenic relative to estrogenic sex hormone levels (b) greater measures of android body fat and (c) lower measures of bone density.

Purposes of Study:

The purposes of this study are therefore:

1. To investigate the nature of the differences in sex hormone levels between pre-menopausal smokers and nonsmokers.

2. To investigate the nature of the differences in body fat distribution between pre-menopausal smokers and nonsmokers.

3. To investigate the nature of the differences in bone density between pre-menopausal smokers and nonsmokers.

4. To clarify the nature of the relationship between the sex hormones, body fat distribution and bone density, and understand how smoking affects this relationship.

5. To implicate a hormonal effect of smoking as a mechanism by which smoking is associated with android body fat and low bone density.

Methods:

Participants in this study will consist of two groups of healthy pre-menopausal females recruited from the local community. All participants must be between the ages of 20-35 years. One group will consist of smokers, the other of nonsmokers.

To obtain the information needed to resolve this study, the following measurements will be made on all participants. They need not be made on the same day. A total time commitment of approximately two hours is required.

Anthropometry: Anthropometric measurements will be made at the John Labatt High Performance Laboratory, Sport & Exercise Sciences Research Institute, Max Bell Centre, Fort Garry Campus, University of Manitoba. The entire procedure will take about 1 hour. Height, weight, several skinfold measurements, girths, bone breadths and lengths will be done. These measurements will allow the estimation of the volumes of adipose tissue, muscle and bone in your body. Measurements will be made in triplicate and size-adjusted sum of skinfolds will be used as an index of fatness. Women should wear a 2-piece bathing suit or loose fitting running shorts and T-shirt or halter top. This procedure will be performed in the late afternoon or evening in a post-absorptive state. Salty food and extremes of over or under-hydration must be avoided.

Bone Density Measurements: Bone density determination will be made at the department of Nuclear Medicine, St. Boniface General Hospital. This procedure requires that you be exposed to a small amount of radiation (less than a chest x-ray). Measurements will include a scan of your total body from head to toe and, more specifically, the vertebrae in your lower back (vertebrae L1-L4), and of the hip (head and neck of the femur) on your right side. These bone density measurements will allow estimation of the bone mineral content of your body. For the total body scan you will be asked to lay on your back for about 20 minutes. For the spine scan you will be asked to lay on your back with your legs raised on a support; this procedure will take about 7 minutes. For the hip scan you will again lay on your back but with your right foot in a support; this procedure takes about 7 minutes. Wear loose fitting, comfortable clothing without metal buttons or zippers.

Sex Hormone Levels: Serum levels of two sex hormones, estradiol (an estrogen) and testosterone (an androgen) will be determined. In order to do this a small amount of blood (10 ml) must be obtained. Blood will be taken from a superficial arm vein by a Registered Nurse the same time that bone density is determined at St. Boniface General Hospital. The procedure is relatively painless and over in a few minutes. This procedure must be performed the morning after an overnight fast and after abstinence from alcohol for at least five days. Actual assays for sex hormone levels will be conducted at the Endocrinology and Metabolism Laboratory, Section of Endocrinology and Metabolism, Health Sciences Centre. (You personally will not be required to go to the Health Sciences Centre.)

Risks and Discomforts:

Assessment of skinfold thicknesses by application of skinfold caliper may cause mild discomfort.

Anthropometrical measurements in general may cause psychological discomfort in that minimal clothing must be worn during these procedures.

Possible risks which may be incurred while blood is being obtained are moderate bruising or, rarely, fainting.

You will be exposed to a small amount of radiation during the assessment of bone mineral density. The dosage you will receive is about 1/20th that of a standard chest X-ray -- about the same amount of radiation you might be exposed to if you flew from Vancouver to Toronto.

For all procedures, trained personnel will conduct the testing, and emergency procedures have been outlined to deal with any unusual situation which may arise.

This study has received ethical approval from the University of Manitoba Committee on Research Involving Human Subjects.

Data Collection:

You are assured that data collected by this investigation will remain confidential with regard to your identity. Should this research be published confidentiality will be strictly maintained. No non-coded information will be disclosed to third parties. Upon completion of research all non-essential materials accumulated during the study will be safely disposed of; those which are essential are assured continued security to maintain confidentiality.

Subject Rights:

You have the right to withdraw participation completely or partially at any time. Each participant will be provided with their own results following testing. Any questions, comments, or criticisms may be directed to Dr. Alan D. Martin, Director, or Dr. Don T. Drinkwater, Research Scientist, Sport & Exercise Sciences Research Institute, University of Manitoba, 474-8629 or 474-8646, respectively.

Please Note: Mark Daniel may be contacted at 474-9747, 474-8773, or 474-8647 (University of Manitoba) or 775-8420 (home).

Sport & Exercise Sciences Research Institute
University of Manitoba

Consent Form

Smoking, Adipose Tissue and Bone Mineral Density Study

Name: Sex: Age:

I hereby authorize Mark Daniel or other competent personnel to perform the following procedures and investigations:

1.) administer a Subject Screening Questionnaire
2.) draw a blood sample for sex hormone assays
3.) obtain measures of anthropometrical variables
4.) determine bone mineral density

I have read the description of the study in the document entitled "Smoking, Adipose Tissue and Bone Mineral Density Study: Information to Participant."

I understand the purpose of the study and the procedures which will be employed. I also understand the possible risks and discomforts which may be involved. I am aware of my rights as a subject; specifically, that I may withdraw my consent and terminate my participation at any time.

I have had ample opportunity to ask questions regarding the nature, procedures, risks and benefits of this study, and I have had all such questions answered to my satisfaction.

Date:

Subject's Signature:

Witness's Signature:

APPENDIX C

Subject Screening Questionnaire

APPENDIX C

Subject Screening Questionnaire

Sport & Exercise Sciences Research Institute
University of Manitoba

Smoking, Adipose Tissue and Bone Mineral Density Study:
Subject Screening Questionnaire

This questionnaire is concerned with determining your eligibility for participation in this study. It is important that you consider each question carefully and answer truthfully. The first section concerns descriptive personal information which must be obtained so that you can be contacted. The second section concerns medical information regarding your current state of health. The third section is concerned with eliciting information directly relevant to your status within this study.

Section I: Personal Information

Full name: _____
(Surname) (Given) (Middle)

Date of Birth: _____
(Day) (Month) (Year)

Address: _____
(Street Number) (Street)

_____
(City) (Postal Code)

Telephone Number: _____
(Home) (Business)

Section II: Health Status

1) Has a doctor ever said you have heart trouble? Y / N

2) Do you frequently have pains in your heart and chest? Y / N

3) Do you often feel faint or severely dizzy? Y / N

4) Has a doctor ever said your blood pressure was too high? Y / N

5) Are you currently pregnant? Y / N

6) Do you or are you taking any prescription or non-prescription drugs on a regular basis? Y / N

If you answered "YES" to this question, then please indicate:
(a) the name of the drug: _____

(b) what you take it for: _____

_____

II:

(please leave this section blank)

Section III: Study Status
Part A: Smoking Status

7) Are you a smoker or nonsmoker? smoker / nonsmoker
(Please Circle)

If you are a smoker please answer Question (8) and then go to Part B

If you are a nonsmoker, please answer Questions (9) and (10)

8) You are a "smoker".
(a) Please indicate, on the average, how many cigarettes you smoke per day:

_____

(b) How long have you smoked the above number of cigarettes per day?

_____

(c) How long have you been a smoker?

_____

9) You are a "nonsmoker". Have you ever smoked? Y / N

If you answered "YES" to this question, then please estimate:
(a) how long it has been since you quit smoking: _____

(b) the length of time that you smoked for: _____

(c) the average number of cigarettes you smoked per day: _____

10) Are you exposed to "second-hand" smoke? Y / N

If you answered "YES" to this question, then please estimate:
(a) the average length of time per day that you are exposed:

_____

(b) the intensity of your exposure:

low low/moderate moderate moderate/high high
(Please circle a category)

III. A:

(please leave this section blank)

Part B: Status of Oral Contraceptive Use

11) Do you currently use oral contraceptives ("the pill")? Y / N

12) Have you ever used oral contraceptives? Y / N

If you answered "YES" to this question, then please estimate:
(a) how long you took them for: _____

(b) how long it has been since you stopped using them:

_____

III. B:

(please leave this section blank)

Part C: Status of Alcohol Consumption

13) Do you consume alcohol? Y / N

If you answered "YES" to this question, then please estimate, on the average:
(a) how many 1 oz. drinks of alcohol, or equivalent (eg. 1 oz. alcohol = 12 oz. beer = 4 oz. wine), you consume per week?

_____

III. C:

(please leave this section blank)

Part D: Acute Weight Change Status

14) Have you undergone an acute (relatively fast) change in weight (gain or loss) within the six months? Y / N

If you answered "YES" to this question, then please estimate:
(a) the amount of weight gained or lost: _____

(b) the time span of this change: _____

III. D:

(please leave this section blank)

Part E: Status of Physical Activity

15) Are you physically active? Y / N

If you answered "NO" to this question, then please go to Part F and continue with Question (20).

If you answered "YES" to this question, then please continue on through Part E.

16) You are physically active. Please indicate the nature of your activities:

17) Please indicate the number of hours that you train or are physically active per week:

18) Please estimate the average intensity of your training:

low low/moderate moderate moderate/high high

(Please circle a category)

19) Are you a competitive athlete? Y / N

If you answered "YES" to this question, then please

(a) indicate the level of competition (eg. amateur, professional):

(b) describe the nature of the competition (if different from that noted in (16):

III. E:

(please leave this section blank)

Part F: Parity Status

20) Please indicate the number of times you have given birth:

III. F:

(please leave this section blank)

Part G: Menstrual Status

21) Is your menstrual cycle regular? Y / N

22) Please estimate (in days) the average length of your menstrual cycle:

_____

23) Please estimate (in days) the average length of your menstrual flow:

_____

24) How many menstrual cycles do you have per year?

_____

25) Please indicate:
(a) your age at menarche (ie. your first menstrual period): _____

(b) the year _____ and month _____ that menarche occurred.

26) Have you ever missed a menstrual period or periods (excluding pregnancy)? Y / N

If you answered "YES" to this question, then please indicate:
(a) when this occurred: _____

_____

(b) the number of consecutive periods you missed:

_____

27) Have you ever been told by a doctor that you are oligo-ovulatory, have short luteal phase, polycistic ovarian syndrome, or any other menstrual disorder? Y / N

If your answer to this question is "YES", then please clarify:

_____

III. G:

(please leave this section blank)

Thank-you for completing this questionnaire. You are assured that this information will remain strictly confidential. You will be notified in the near future as to whether or not you are a suitable subject for this study.

APPENDIX D

Anthropometric Proforma

APPENDIX D

Anthropometric Proforma

Sport & Exercise Sciences Research Institute
University of Manitoba

Anthropometric Proforma - Smoking, Adipose Tissue and Bone Mineral Density Study

Name_____ M / F ID#_____
(last) (first & initial) (circle one)

Birth Date _____/_____/_____ Measurement Date _____/_____/_____
(month / day / year) (month / day / year)

Measured by _____ Smoker / Nonsmoker
(circle one)

Body Size:

height [stature] (cm)				
weight (kg)				

Skinfolds (mm):

biceps				
triceps				
subscapular				
iliac crest				
supraspinale				
abdominal/umbilical (vertical)				
front thigh				
medial calf				
_____				
_____				

Girths (cm):

arm				
forearm				
wrist				
chest				
waist				
abdominal (umbilical)				
gluteal				
upper thigh				
mid thigh				
calf				
_____				

Breadths (cm):

biacromial				
bi-iliac				
humerus				
wrist				
femur				
ankle				
_____				
_____				
_____				

Direct Lengths (cm):

arm (humerus)				
forearm (radius)				
thigh (femur)				
leg (tibia)				
foot length				
_____				
_____				
_____				

Notes:

bust _____

_____

_____

facial hair (colour, amount) _____

_____

_____

body hair (colour, amount) _____

_____

_____

fat pouches (trochanteric, abbuctor region) _____

_____

_____

Observations:

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