EFFECTS OF A LOW-INSULIN-RESPONSE, ENERGY-RESTRICTED DIET ON WEIGHT LOSS AND ENDOCRINOLOGICAL PARAMETERS IN OBESE, ANOVULATORY WOMEN IN THEIR REPRODUCTIVE YEARS

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Abbreviations

A4	Androstenedione
ACTH	Adrenocorticotrophic hormone
AUC	Area under the curve
BIA	Bio-electrical impedance assessment
BMI	Body mass index
CRF	Corticotrophin releasing hormone
DHEA	Dehydroepiandostenedione
DHEAS	Dehydroepiandosterone sulphate
DHT	Dehydrotestosterone
E1	Estrone
E2	Estradiol
FSH	Follicle stimulating hormone
FT4	Thyroxin
G:I	Glucose-to-insulin ratio
GI	Glycemic index
GIP	Gastric inhibiting peptide
GnRH	Gonadotrophin releasing hormone
GR	Glucose response
HDL	High density lipoprotein
IGF	Insulin-like growth factor
IGFBP	Insulin-like growth factor binding protein
II	Insulin index
IR	Insulin response
IS	Insulin score
LH	Leutinizing hormone
LID	Low-insulin response, energy restricted diet
NB	Non-parboiled
ND	Normal balanced, energy restricted diet
PAI-1	Plasminogeen activator inhibitor-1
PCOS	Polycystic ovarian syndrome
SHBG	Sex hormone-binding globulin
T	Testosterone
TB	Parboiled
WHR	Waist-to-hip ratio

CHAPTER 1

INTRODUCTION

1.1 Introduction and problem statement

There is consistent evidence that obese woman are less fertile than women of normal body weight. This phenomenon is observed with regard to disorders of normal pregnancy rates, spontaneous ovulation, *in vitro* fertilisation and ovulation induction (Guzick *et al.*, 1994; Galtier-Dereure *et al.*, 1997). Obesity, in particular android obesity, is associated with several sex steroid abnormalities in pre menopausal women including (I) increased free estrogen and androgen fractions, (2) decreased sex hormone-binding globulin (SHBG), and (3) increased bioactive oestrogen delivery to target tissue (Ricardo *et al.*, 1997, p460).

The state of insulin resistance with secondary hyperinsulinemia is commonly observed in obese, infertile women (Barbieri *et al.*, 1988; Caro, 1991). The gonadotropic effects of insulin on ovarian steroid hormone synthesis were shown *in-vivo* and *in-vitro* (Poretsky & Kalin, 1987; Barbieri *et al.*, 1988; Nestler *et al.*, 1989). Insulin can directly stimulate androgen production by the ovarian stroma (Barbieri *et al.*, 1986). Furthermore, it was found that insulin and insulin-like growth factors I (IGF-I) augment luteinizing hormone-stimulated androgen biosynthesis in rat ovarian theca cells (Cara & Rosenfield, *1988*; Cara *et al.*, 1990). The exaggerated insulin action on the ovarian tissue may present the pathological mechanism for the disturbances of the endocrine profile and menstrual cycle, hence to infertility in some obese women (Insler *et al.*, 1993).

Weight loss is associated with a significant improvement in menstrual abnormalities, ovulation and fertility rates with a reduction in hyperandrogenism and hyperinsulinemia (Hollman *et al.*, 1996; Pasquali *et al.*, 1997). A reduction in insulin concentrations by diet has been demonstrated to reduce ovarian androgen production (Nestler & Jabukowitz, 1996). It is suggested that weight loss should be the first option in the treatment of overweight infertile women due to considerable cost savings (Kopelman *et al.*, 1980; Gerhard & Postneek, 1988; Zumoff, 1988; Pasquali *et al.*, 1989; Clark *et al.*, 1995; Pasquali *et al.*, 1997; Clark *et al.*, 1998)

Ricardo *et al.* (1997, p460) however, remark that most of the abnormalities associated with obesity can be improved by reducing body weight. However, this approach remains one of the most unsuccessful therapeutic objectives in clinical practice especially in the long term.

Pasquali *et al.* (1997) state that theoretically diet could play a role in the development of the obesity-polycystic ovarian syndrome (PCOS). The PCOS is characterized by chronic anovulation, elevated androgen concentrations and polycystic ovaries. It is also associated with metabolic disturbances, e.g. insulin resistance with compensatory hyperinsulinemia (Duanif *et al.*, 1988). Data suggest that women eating a vegetarian-rich and fibre-rich diet have lowered androgen blood concentrations compared to those women following a typical Western diet (Hill *et al.*, 1980). Rose *et al.* (1991) state that a high-fibre diet reduces oestrogen concentrations in premenopausal women, and Lefebvre *et al.* (1997) suggest that a low-fibre, high lipid intake may increase estrogens and androgens. Moreover, some authors have described a very high lipid intake in women with polycystic ovarian syndrome and there are studies reporting a negative correlation between lipid intake and SHBG values (Wild *et al.*, 1985).

The fact that weight loss significantly improves hyperandrogenism is well-documented by several authors (Pasquali *et al.*, 1989; Kiddy *et al.*, 1992; Guzick *et al.*, 1994, Clark *et al.*, 1995, Clark *et al.*, 1998). A moderate weight loss (<5%) may restore menstrual function, thus indicating that energy restriction can be more important than weight loss (Lefebvre *et al.*, 1997). Lefebvre *et al.* (1997) further state that it is of interest to underline the impact of diet on hyperinsulinemia irrespective of the weight loss. This question was highlighted by Slabber *et al.*, (1994) who compared the effects of two low-energy diets on serum insulin concentrations and weight loss in 30 obese hyperinsulinemic females over a 12-week period. The first diet was designed to evoke a low insulin response (low-insulin-response diet-LID), and the second one was a normal balanced energy restricted diet (ND). Mean weight was significantly reduced after both LIRD and ND but fasting insulin concentrations decreased more after LID compared with ND.

Numerous studies have revealed that the combination of carbohydrate-rich and protein-rich foods in the same meal increases the postprandial insulin response (Rabinowitz *et al.*,1966; Nuttall *et al.*, 1984; Simpson *et al.*, 1985; Krezowski *et al.*, 1986; Spiller *et al.*, 1987; Gulliford *et al.*, 1989). Other factors that influence the postprandial rise in blood insulin are those affecting the rate of carbohydrate digestion and absorption, which include the chemical composition and physical form of ingested starch (Behall *et al.*, 1989; Englyst *et al.*, 1987; Wolever *et al.*, 1990), processing method (Ross *et al.*, 1987; Jenkins *et al.*, 1987), presence of viscous fibre (Wolever *et al.*, 1990; Jenkins *et al.*, 1987) and anti-nutrients in the food or meal consumed (Golay *et al.*,1991). Slabber *et al.* (1994) designed the low-insulin-response diet to evoke low responses to insulin, taking its account the response of insulin to common components in foods and their combinations.

Intervention studies suggested that reducing weight and/or hyperinsulinemia either by diet alone or by a combination of diet and drugs may improve the hormonal and metabolic profiles of obese women with PCOS (Lefebvre *et al.*, 1997). Furthermore, Pasquali *et al.* (1997) stated that dietary manipulations with specific regard to insulin-lowering regimens in the treatment of obese infertile women must be investigated.

The following question arose from a dietetic point of view:

Can a low-insulin-response, energy-restricted diet have a positive effect on endocrinological parameters in obese women with menstrual problems taking into account the gonadothropic function of insulin and the insulin-lowering effect of the diet, and how does this effect compare with the conventional energy restricted diet?

1.2 Objective

The main objective of this study is to determine the effect of a low-insulin-response, energy restricted diet designed by Slabber *et al.* (1994) on weight loss and endocrinological parameters in the treatment of obese females with menstrual problems and infertility.

The following specific objectives were formulated:

- To determine the effect of the LID on weight loss and endocrinological parameters in obese hyperinsulinemic and non-hyperinsulinemic females.
- To determine the effect of a ND on weight loss and endocrinological parameters in obese hyperinsulinemic and non-insulinemic females.
- To compare the effects of the LID with the ND with regard to weight loss and endocrinological parameters.
- To compare the compliance with and the acceptance (hunger sensation and the willingness to follow the diet in the future) of the LID and the ND.

1.3 Layout of dissertation

The dissertation is divided into six chapters. Chapter 1 consists of a short introduction which includes the problem statement, motivation and objectives of the study.

The literature on obesity, reproduction and hyperinsulinemia is discussed in Chapter 2, as well as the dietary treatment of obesity and the food factors that influence insulin responses.

Chapter 3 describes the methods used to plan, implement and monitor the study as well as the statistical method for data analyses. Furthermore, the problems experienced during the study are discussed as well as how these problems were solved.

The results of the study are described in Chapter 4, and are represented in tables. The results are discussed in Chapter 5. The results of this study are compared with those of other studies and possible explanations for the results are given.

In Chapter 6 the conclusion is drawn, recommendations for the possible use of the LID as well as future avenues of investigation are discussed. The dissertation is followed by a short summary of the study.

CHAPTER 2:

LITERATURE STUDY

2.1 Introduction

This chapter focuses on the confounding impact of obesity on reproduction with specific regard to insulin resistance and hyperandrogenism as well as the dietary treatment of obesity.

2.2 Reproductive hormone biosynthesis, metabolism and mechanisms of action

The human ovary produces three classes of sex steroids: estrogens, progestins and androgens (Speroff, 1999, p39) whereas testosterone and dihydrotestosterone are the main androgens of the testes (Meyer, 1996, p1910). Estrogens and progestins are the female sex hormones. Estrogen secretion is mainly regulated by follicle-stimulating hormone (FSH), whereas FSH is regulated by circulating FSH, but mainly by circulating estrogen via negative feedback. Leutinizing (LH) hormone regulates male and female sex hormone secretion, thus estrogens, androgens and progestins (Meyer, 1996, p1919). The main functions of estrogen are:

- To stimulate follicle development and ovulation.
- To stimulate the proliferation of the epitheal cells of the cervix, vagina and uterus.
- To stimulate the development of milk glands.
- To stimulate the retention of sodium and water.
- It is responsible for the integrity of female bone health by increasing calcium and phosphorus retention (Meyer, 1996, p1921).

The functions of progesterone include:

- The preparation of the uterus for the implantation of the fertilised ovum.
- To stimulate the development of alveoli in the milk gland during pregnancy.
- To increases the basal body temperature.
- To suppress ovulation and reduce uterus sensitivity to prostaglandins (Meyer, 1996, p1921).

2.2.1. Two-cell system

The two-cell system is a logical explanation of the events involved in ovarian follicular steroidgenesis (Erickson, 1996). The following facts are important in the two cell system: FSH receptors are present in ovarian granulosa cells and FSH receptors are induced by FSH itself. LH receptors are present on the theca cells and initially absent on the granulosa cells, but, as follicles grow, FSH induces the appearance of LH receptors on the granulosa cells. FSH induces aromatase enzyme activity in granulosa cells and all the above mentioned factors are modulated by autocrine and paracrine factors secreted by the granulosa and theca cells (Speroff, 1999, p43).

The initial change from pre-mordial follicle to preantral follicle is independent of hormones, and the stimulus governing this initial step of growth is unknown. Continued growth of the follicle is, however, dependent on FSH stimulation. As the granulosa responds to FSH, proliferation and growth are associated with an increase in FSH receptors, a specific effect of FSH itself, but an action enhanced by the autocrine and paracrine peptides. The theca cells are characterized by steriogenic activity in response to LH, specifically resulting in androgen production. Aromatisation of androgens to estrogens is a distinct activity within the granulosa layer induced by FSH. Therefore, androgens produced in the theca layer must diffuse into the granulosa layer. In the granulose layer they are converted to estrogens, and the increase of estrogens in the peripheral circulation effects the release of the estrogens back toward the thecal layer into blood vessels (Sperroff, 1999, p44).

The thecal and granulose cells secrete peptides that operate as both autocrine and paracrine factors (Kol *et al.*, 1995). IGF is secreted by the theca and enhances the LH stimulation of androgen production in the thecal cells as well as FSH-mediated aromatisation in the granulosa (Voutilainen *et al.*, 1996).

2.2.2 Insulin-like growth factors (IGF's)

IGF's are polypeptides that modulate cell proliferation and differentiation, operating through the binding to specific cell membrane receptors. IGF, also called somatomedins, are peptides that are structural and functionally similar to insulin and that mediate growth hormone action (Guidice, 1992). IGF-I and IGF-II are single chain polypeptides. IGF-I mediates the growth-promoting actions of growth hormone. The majority of circulating IGF-I is derived from growth hormone-dependent synthesis in the liver. However, IGF-I is synthesised in many tissues where production can be regulated in conjunction with growth hormone or independently by other factors (Speroff, 1999, p214).

There are six known insulin-like growth factors binding proteins (IGFBP), IGFBP-1 to IGFBP-6. These binding proteins carry IGF's in serum, prolong half-lives, and regulate tissue effects of the IGF's. (Speroff, 1999, p214). The IGFBP binds IGF-I and IGFBP-II binds IGF-II. IGF-I also binds to the insulin receptor but with low affinity. Insulin binds to the IGF-I receptor with moderate affinity. The IGF-I receptor and the insulin receptor are similar in structure. Ovarian stromal tissue contains IGF-1 receptors (Speroff, 1999, p215).

According to animal studies, both IGF-I and IGF-II are secreted by granulosa cells. IGF-I amplifies the actions of gonadotropins and co-ordinates the functions of the theca and granulosa cells. IGF-I receptors on the granulosa are increased by FSH and LH is augmented by estrogen. In the theca, IGF-I increases steroidgenesis. In the granulosa, IGF-I is important for the formation and increase in numbers of FSH and LH-receptors, steroidgenesis, the secretion of inhibin, and oocyte maturation (Speroff, 1999, p 87).

IGF-I has been demonstrated to stimulate the following in ovarian thecal and granulose cells: DNA synthesis, steroidgenesis, aromatase activity, LH receptor synthesis and inhibin secretion. IGF-II stimulates granulosa mitosis. In the human ovarian cells, IGF-I, in synergy with FSH, stimulates protein synthesis and steroidgenesis. After LH receptors appear, IGF-I enhances LH-induced progesterone synthesis and stimulates proliferation of granulose-luteal cells. IGF-I, in synergy with FSH, is very active in stimulating aromatase activity in preovulatory follicles. Thus, IGF-I can be involved in both estradiol and progesterone synthesis (Speroff, 1999, p215).

2.2.3 Blood transport of steroids

While circulating in the blood, most of the principle sex steroids, estradiol and testosterone are bound to a protein carrier, known as sex hormone-binding globulin (SHBG) produced in the liver. SHBG is a glucoprotein that contains a single binding site for androgens and estrogens. Another 10-30% is loosely bound to albumin, leaving only 1% unbound and free. A very small percentage also binds to corticosteroid-binding globulin. Hyperthyroidism, pregnancy, and estrogen administration increase SHBG levels, whereas corticoids, androgens, progestins, growth hormones, insulin and IGF-I decrease SHBG (Speroff, 1999, p45).

The circulating level of SHBG is inversely related to weight, thus a significant weight gain can decrease SHBG and produce important changes in unbound levels of sex steroids. Another very important mechanism for the reduction of SHBG levels is insulin resistance and hyperinsulinemia (independent of age and weight) (Preziosi *et al.*, 1993). Thus, an increase in insulin in circulation and lower SHBG concentrations may be the mechanism for the impact of weight gain on SHBG. This relationship between the level of insulin and SHBG is so strong that SHBG concentrations is an important marker for insulin resistance, and a low level of SHBG is a predictor for the development of type 2 diabetes mellitus (Linstedt *et al.*, 1991). The distribution of body fat has strong effect on SHBG concentrations. Android or central obesity is associated with hyperinsulinemia, hyperandrogenism and a decrease in SHBG (Peiris *et al.*, 1989).

The biological effects of the major sex steroids are largely determined by the unbound portion, known as the free hormone. In other words, the active hormone is unbound and free, whereas the bound hormone is relatively inactive (Speroff, 1999, p46). The hormone-protein complex may be involved in an active uptake process as the target cell plasma membrane (Rosner, 1990). The albumin-bound fraction of steroids may also be available for cellular action because this binding has a low affinity (Speroff, 1999, p46).

2.2.4 Metabolism of estrogens, progesterone and androgens

Androgens are common precursors of estrogens. The activity of the enzyme 17-beta-hydroxysteroid dehydrogenase converts androstenedione to testosterone, which is not a major secretory product of the normal ovary. It is rapidly demethylated at the C-19 position and aromatised to estradiol, the major estrogen secreted by the human ovary. Estradiol also originates to a large extent from androstenedione via estrone, and estrone itself is secreted in significant daily amounts. Estriol is the peripheral metabolite of estrone and estradiol, and is not a secretory product of the ovary. The formation of estriol is typical of general metabolic detoxification conversion of biologically active materials to less active forms (Speroff, 1999, p46).

Pheripheral conversion of steroids to progesterone is not found in non-pregnant females; rather, the progesterone production rate is a combination of secretion from the adrenal and the ovaries. Including the small contribution from the adrenal, the blood production rate of progesterone in the pre-ovulatory phase is less than 1mg/day. During the luteal phase, production increases to 20 - 30mg/day. The metabolic rate of progesterone, as expressed by its many excretion products, is more complex than estrogen. About 10 – 20% of progesterone is excreted as pregnanediol. Pregnanediol is the chief urinary metabolite of 17-alpha-hydoxyprogesterone (Speroff, 1999, p49). The major androgen products of the ovary are dehydroepiandrostenedione (DHEA) and androstenedione (and only a little testosterone), which are secreted mainly by stromal tissue derived from theca cells. Dehydrotestosterone (DHT) is largely metabolised intracellularly; hence, the blood DHT is only about one-tenth the level of circulating testrosterone, and it is clear that testosterone is the major circulating androgen. In tissues sensitive to DHT, only DHT enters the nucleus to provide the androgen message. DHT can perform androgenic actions within cells that do not possess the ability to convert testosterone to DHT. The metabolite of androstenedion, 3alpha-keto-androstenedione glucuronide, is the major metabolite of DHT and can be measured in the plasma, indicating the level of activity of target tissue conversion of testosterone to DHT (Speroff, 1999, p51).

2.2.5 Excretion of steroids

Active steroids and metabolites are excreted as sulpha and gluco conjugates. Conjugation is done by the liver and intestinal mucosa, and is the first step in deactivating the steroids which is essential for excretion into the liver and bile (Speroff, 1999, p52).

2.3 The menstrual cycle

The menstruation cycle consists of three phases namely the menstruation phase, the follicular phase and the lutheal phase. The first day of menstruation is normally regarded as the first day of the cycle and the last day before menstruation resumes is regarded as the last day of the cycle (Meyer, 1996, p1925).

The menstruation phase can last from three to seven days and is initiated due to the degeneration of the corpus luteum. The follicular phase begins 3 to 5 days after the onset of menstruation with the development of 10 – 15 primary follicles. Of these follicles only one will develop as a mature follicle. FSH stimulates the development of the follicles while granulosa of the follicles secrete estradiol that stimulates the recovery of the endometrium. The follicular phase is completed by day 13 – 14 with a sharp increase in FSH, estrogen and LH concentrations. This results in ovulation while LH stimulates the development of the corpus luteum. Estradiol and progesterone are secreted by the corpus luteum. LH levels decreases by day 25 –26 of the menstrual cycle if the ovum is not fertilised and this leads to the degeneration of the corpus luteum which will initiate the menstruation phase. If conception has occurred the corpus luteum will secrete progressively more estradiol and progesterone up to day 70 – 90 until the placenta continues to produce estradiol and progesterone. In pregnancy the function of the corpus luteum is regulated by human chorionic gonadotrophin (Meyer, 1996, pp1925 - 1927).

2.4 Obesity and reproduction

2.4.1 Obesity and the reproductive system in females

Obesity produces a variety of abnormalities in the female reproductive system (Bray, 1997). The onset of menarche is earlier in obese girls than in girls of normal body weight. One explanation for this phenomenon was proposed by Frisch and Revelle (1971). It is based on the observation that menstruation is initiated when body weight reaches a 'critical mass'. As growth rate accelerates in late childhood, the percentage of body fat rises and initiates the pubertal process (Frisch and Revelle, 1971). Because obese girls reach the critical weight at a younger age, menses on average usually occurs a year earlier. When fat loss occurs and drops below this critical range, menstruation frequently disappears as observed in ballet dancers and distance runners (Firsch & Revelle, 1971). Women with hirsutism and anovulatory cycles are on average 14kg heavier than women with no menstrual abnormalities.

Pregnancy influences obesity and vice versa. Obese women tend to have heavier babies and larger placentas. Furthermore, just as the onset of menarche is earlier in obese women, so data suggest that the onset of ovarian failure and increased production of FSH at menopause are four years earlier in obese women than in women of normal body weight (Bray, 1997).

2.4.2 Sex steroid concentrations and metabolism in obese women

Obesity is associated with several abnormalities of sex hormone balance in premenopausal women, the extent of which is proportionate to the degree of excess body weight. These abnormalities of sex steroid balance in premenopausal women, include both androgens and estrogens as well as their main transport protein, sex hormone-binding globulin (SHBG). A functional hyperandrogenism develops and the condition is associated with increased estrogen production, and specific alternations of steroid transport proteins and of several enzyme systems involved in steroid metabolism. (Pasquali, 1997).

Furthermore, adipose tissue is an important site of active steroid production and metabolism. It possesses the aromatase enzyme which allows a fraction of the circulating androgens, androstenedione (A4), and testosterone (T), to be converted to the estrogens estrone (E1) and estradiol (E2), respectively (Mendelson *et al.*, 1989). Adipose tissue contains other enzyme systems such as 17B-hydroxysteroid dehydrogenase which catalyses the transformation of E2 to E1 and A4 to T (Perel *et al.*, 1979). Body fat therefore, appears to be an important tissue where androgens and estrogens undergo active metabolism and formation (Pasquali, 1997).

2.4.2.1 Sex hormone-binding globulin (SHBG)

Circulating SHBG concentrations are inversely proportional to body weight (Glass, AR., 1989). Body fat distribution is also important in determining SHBG in obese women. Women with central (android) obesity usually have lower SHBG concentrations compared to their age- and weight-matched counterparts with genoid (peripheral) obesity (Pasquali *et al.*, 1993). The concentration of SHBG is regulated by a complex of hormones which includes estrogens, iodothyronines, and growth hormones as stimulatory factors, as well as androgens and insulin as inhibitory factors (von Shoultz *et al.*, 1989). *In vitro* studies have shown that insulin inhibits hepatic SHBG synthesis (Evans *et al.*, 1983; Plymate *et al.*, 1988; Nestler *et al.*, 1989; Haffner *et al.*, 1993). Suppression or stimulation of insulin secretions *in vivo* has been found to be inversely associated with changes in SHBG in hyperandrogenic obese women (Plymate *et al.*, 1988; Nestler *et al.*, 1989). Reduced SHBG concentrations are therefore commonly associated with obesity (in particular android obesity), type 2 diabetes mellitus, hyperandrogenic states such as polycystic ovarian syndrome (PCOS) and cardiovascular atherosclerotic disease (Poretsky, 1991). PCOS is characterized by chronic anovulation, abnormal gonadothropin concentrations, elevated androgen concentrations and polycystic ovaries (Lefebvre *et al.*, 1997).

2.4.2.2 Androgens

Although levels of 17-ketosteroids may be higher than normal in obese women (Glass, 1989), levels of the main androgens are usually high only in obese women with amenorrhoea (Zhang *et al.*, 1984) and are normal in obese women with regular menstrual cycles (Glass, 1989; Pasquali *et al.*, 1987). Androgen production and metabolism may also be altered by the pattern of body fat distribution. Due to the reduction in SHBG concentrations, the free T fraction tends to be higher in women with central obesity compared to those with peripheral obesity (Evans *et al.*, 1983). The reduction in SHBG increases the metabolic clearance rate of SHBG-bound steroids such as androgens (Von Shoultz *et al.*, 1989). The metabolism of those steroids not bound to SHBG is also modified by obesity (Pasquali, 1997, p457). Kirschner *et al.*, (1983) examined a group of obese young women and found that the production rate of A4 averaged 50% more than in the nonobese controls.
Kurtz *et al.*, (1987) also observed that the production of DHEA increased 94% in obese women compared to the non-obese controls. In another study by Kirschner *et al.*, (1990) they found that premenopausal women with central obesity had higher T production rates than those with peripheral obesity, whereas no differences were found in A4 or DHT production.

2.4.2.3 Estrogens

Obesity can also be considered as a condition of exaggerated oestrogen production. It has been reported that the conversion of androgens to estrogens in peripheral tissues is significantly correlated with body weight and the amount of body fat (Siiteri, 1981). Due to the reduced SHBG synthesis and lower circulating SHBG concentrations in obesity the free E2 fraction increases, thus increasing exposure of target tissues to this hormone. Moreover, the metabolism of estrogens is altered in obese women. A higher than normal production of E1 sulphate is observed due to the reduction in its metabolic clearance and increased production rate. This results in an overall increase in the active oestrogen fraction, particularly E1 in several tissues, including the hypothalamus, cerebral cortex and endometrium. The final result of these metabolic disturbances on oestrogen balance is an increased ratio of active to inactive estrogens in obese women (Pasquali, 1997).

2.5 The role of body fat distribution in androgen excess

Overweight, anovulatory women with hyperandrogenism have a characteristic distribution of body fat known as android obesity (Peiris *et al.*, 1989). Android fat distribution is associated with hyperinsulinemia, impaired glucose tolerance, diabetes mellitus, and an increase in androgen production rates resulting in decreased levels of sex hormone-binding globulin and increased levels of free testosterone and estradiol (Kirchner *et al.*, 1990; Pasquali *et al.*, 1991).

Givens (1991) states that the main determining factor influencing reproduction is not the quantity of fat in obese women but rather the localization of the excess fat. Women with abdominal body fat distribution have higher concentrations of LH and androstenedione than women with peripheral body fat distribution (Pasquali *et al.*, 1994). Increased androgen activity is more frequent in women with upper body obesity than in women with lower body obesity (Kirschner *et al.*, 1990).

2.5.1 Pathophysiology of the modulatory effects of body fat distribution on androgen excess

The major mechanism by which body fat distribution may modulate androgen excess and its disorders are (i) abnormalities in gonadotropin secretions (primarily LH); (ii) increased adrenal production of androgens via activation of the corticotrophin-releasing hormone (CRF)- adrenocorticotrophic hormone (ACTH)- adrenal axis; (iii) modulation of serum concentrations of sex steroids and SHBG and (iv) alternations of the insulin / IGF-I system (Pasquali, 1997).

2.5.1.1 Modulation of circulating androgens and SHBG concentrations

Obese girls with android obesity have higher serum testosterone levels and free androgen index, and lower SHBG compared to obese girls with genoid obesity (Wabitsch *et al.*, 1995). In this study waist-to-hip-ratio (WHR) but not BMI or percent body fat correlated positively with testosterone and free androgen index (Wabitsch *et al.*, 1995). According to Pugeat *et al.* (1991) the predominant mechanism whereby body fat distribution may affect SHBG: central fat distribution is associated with insulin resistance and elevated circulating insulin concentrations, and this hyperinsulinemia, in turn, suppresses hepatic SHBG production.

2.5.1.2 Increased adrenal production of androgens

There is evidence to prove that women with hyperandrogenism and PCOS reveal increased adrenal secretion of cortisol (Rodin *et al.*, 1992).

Both obese women and obese adolescent girls with an increased WHR have low morning plasma cortisol levels (Wabitsch *et al.*, 1995; Marin *et al.*, 1992), yet there is a positive correlation between WHR and urinary excretion of cortisol in obese women (Marin *et al.*, 1992). An explanation of these findings may be that the metabolic clearance rate of cortisol is increased due primarily to an elevated number of glucocorticoid receptors in the expanded adipose tissue mass (Rebuffe *et al.*, 1990). Thus, reduction of negative feedback by cortisol on pituitary ACTH release could activate the hypothalamic-pituitary-adrenal axis (Pasquali *et al.*, 1993) and account for the elevated concentration of dehydroepiandosterone sulfate (DHEAS) frequently observed in women with polycystic ovarian syndrome (PCOS) and central obesity (Wabistch *et al.*, 1995).

2.6 The role of nutritional factors on hormonal parameters

Nutritional factors may interfere with insulin secretion and sensitivity (Lefebvre *et al.*, 1997). Both PCOS and binge eating are common disorders of the female population and some studies report an association between abnormal eating behaviour and disovulation or PCOS (Pirke *et al.*, 1986; McCluskey *et al.*, 1991).

There is a complex interrelationship between various nutritional factors and endocrine abnormalities (Lefebvre *et al.*, 1997). Diet is known to play an important role in regulating the metabolism of sex steroids with specific regard to LH (Pirke *et al.*, 1991; Snow *et al.*, 1990). Schneider *et al.*, 1989 and Bronsen *et al.*, (1991) pointed out from animal models that ovulation is dependent on the availability of oxidized metabolic fuels – namely, glucose and fatty acids. For example, food intake influences LH secretion, as shown by the observation that food-restricted female rats exhibited high amplitude LH pulses a few hours after eating their one-daily meal, but not at any other time (Bronson *et al.*, 1991). Loucks and Heath (1994), and Olsen *et al.*, (1995) found that a short four-day dietary restriction affected LH pulsatility in women of normal weight although a major impact on ovulation and menstrual function required a longer period of dieting (Olsen *et al.*, 1995). However, Cameron (1989) indicated that after a six to nine week isoenergetic protein-deficient diet, monkeys maintained normal circulating LH and FSH concentrations suggesting that a deficient protein intake does not provide the signal leading to reproductive impairment in restricted monkeys. Similar studies have reported that neither fat nor carbohydrate deficiencies resulted in a suppression of circulating gonadotrophin concentrations (Cameron, 1989). Extensive animal research supports the view that suppression of the reproductive function during under-nutrition is not due to a deficiency of specific nutrients, but is a result of energy deficiency (Foster and Olster, 1985; Foster *et al.*, 1989; Cameron, 1989).

Pirke *et al.* (1986) found that vegetarian diets with a low protein content disrupted the cycle more than non-vegetarian diets causing the same weight loss. This raises the possible role of specific nutrients on ovulatory regulation in humans. A high-fibre diet reduces serum estrogen concentrations in premenopausal women (Rose *et al.*, 1991) and it is suggested that a low-fibre high-lipid diet may also increase estrogen and androgen pools.

An additional mechanism whereby nutrition may interfere with endocrine abnormalities is represented by its impact on the IGF system. The intra-ovarian IGF system has been implicated in the growth and differentiation of ovarian follicles and this system is linked to the disturbed follicular development in PCOS (Guidice, 1995). Moreover, the IGF system is also found at brain level where it may interfere with the gonadotrophic axis. There is consistent evidence that nutritional status may modify the serum concentrations of IGF and its IGFBP (Thissen *et al.*, 1994). Both energy and protein are critical in the regulation of serum IGF-1 concentrations. In general, dietary restriction decreases IGF-1 and serum IGFBP-3 concentrations while it increases serum IGFBP-1 and IGFBP-2. Obese subjects maintain their IGF-1 during diet restriction and have a tendency to present increased IGF-1 during overfeeding (Thissen *et al.*, 1994). Conover *et al.*, (1992) found that nutritional intake decreases the circulating levels of IGFBP-1 because of the increase in insulin, which then directly inhibits IGFBP production in the liver.

Some researchers suggest the relationship of insulin and glucose, fatty acids, and amino acids as the underlying factor of these parameters on gonadotrophin-releasing hormone (GnRH) pulse generator (Schneider *et al.*, 1989; Bronson *et al.*, 1991). However, in rhesus monkeys, stimulation of LH secretion by food intake does not appear to be mediated by insulin, as demonstrated by the persistent food-induced LH pulses after a suppression of insulin by daizoxide (Schneider *et al.*, 1989).

2.7 Insulin resistance, hyperinsulinemia, and hyperandrogenism

The clinical association of hyperinsulinemia and anovulatory hyperandrogenism is commonly found throughout the world and among different ethnic groups (Osei & Schuster, 1992; Norman *et al.*, 1995).

There are studies indicating that androgens can induce hyperinsulinemia. However, most of the evidence supports hyperinsulinemia as the primary factor, especially the experiments in which turning of the ovary with GnRH agonist does not change the hyperinsulinemia or insulin resistance (Geffner *et al.*, 1986; Dunaiff *et al.*, 1990; Poretsky, L., 1991; DeClue *et al.*, 1991; Grainger *et al.*, 1992).

Hyperinsulinemia and hyperandrogenism, however, are not confined to anovulatory women who are overweight. Increased androgen levels and insulin resistance have been reported in both obese and non-obese anovulatory women (Chang *et al.*, 1983; Dunaif *et al.*, 1989; Poretsky, 1991; Buyalos *et al.*, 1992). However, insulin levels are higher and LH, SHBG, and IGFBP-1 levels are lower in obese women with polycystic ovaries compared to non-obese women with polycystic ovaries (Anttila *et al.*, 1991; Insler *et al.*, 1993; Morales *et al.*, 1996).

There are several mechanisms for the state of insulin resistance including peripheral target tissue resistance, decreased hepatic clearance, or increased pancreatic sensitivity (Poretsky, 1991). Studies with the euglycemic clamp technique have indicated that hyperandrogenic women with insulin resistance have both peripheral insulin resistance, and in addition, a reduction of insulin clearance rate due to decreased hepatic insulin extraction (Poretsky, 1991; O'Meara *et al.*, 1993).

2.7.1 Mechanism whereby insulin could increase ovarian androgen production

There is an important correlation between the degree of hyperinsulinemia and hyperandrogenism (Chang *et al.*, 1983; Dunaif *et al.*, 1989; Buyalos *et al.*, 1992). At higher concentrations insulin binds to Type 1 IGF receptors - those are similar in structure to insulin receptors; both IGF and insulin transmit their signals by initiating tyrosine autophosphorylation of their receptors. Thus, when insulin receptors are blocked or deficient in number, insulin is expected to bind to the type 1 IGF receptors (Fradkin *et al.*, 1989). In view of the known actions of IGF-1 in augmenting the thecal androgen response to LH, activation of IGF-1 receptors by insulin would lead to increased androgen production in thecal cells (Bergh *et al.*, 1993). However, Speroff (1999, p503) emphasises the evidence that the endogenous insulin-like growth factor in the human ovarian follicle is IGF-2 in both the granulose and thecal cells. Studies indicating the activity of IGF-1 with human ovarian tissue can be explained by the fact that both IGF-1 and IGF-2 activities can be mediated by the type 1 IGF receptor, which is structurally similar to the insulin receptor.

Nestler (1997) proposed that an increase in insulin activates a signaling system that operates via inositolphosphoglycan to stimulate steroidgenesis. This pathway would operate by means of insulin binding to its own receptor, a pathway supported by *in vitro* studies of both granulose and thecal cells (Wills & Franks 1995; Wills *et al.*, 1996; Nestler *et al.*, 1998).

There are two other important actions of insulin that contribute to hyperandrogenism in the presence of hyperinsulinemia: inhibition of hepatic synthesis of SHBG and inhibition of hepatic production of IGFBP-1 (Speroff, 1999, p504).

2.7.1.1 Direct effects of insulin on ovarian androgen production

On the surface it may seem paradoxical that insulin should stimulate ovarian androgen production in a woman who is otherwise resistant to insulin. Several theoretical mechanisms can explain how a woman resistant to the effects of insulin on glucose transport could nonetheless remain fully sensitive to insulin stimulation of androgenic pathways (Nestler, 1997).

The most frequently cited possibilities are that insulin could either cross-react with the ovarian IGF-I receptor or bind to hybrid insulin receptors. These explanations appear to be unlikely because (i) the elevation in circulating insulin in PCOS women is usually modest and overlaps substantially with that observed in healthy obese women, and (ii) hybrid insulin receptors have not been identified on human ovaries (Ricardo *et al.*, 1997).

It has also been suggested that insulin could act indirectly by reducing intrafollicular levels of IGFBP-1, thereby increasing intrafollicular concentrations of free IGF-1. IGF-1 is a potent stimulator of LH-induced androgen synthesis by ovarian interstitial cells (Cara & Rosenfield, 1988; Adashi *et al.*, 1992), which may, in part, be due to an induction of LH receptors on these cells by IGF-1 (Cara *et al.*, 1990).
However, this explanation is also unlikely in view of evidence which suggesting that total intrafollicular IGF-binding capacity in PCOS may be increased rather than reduced (Buylos, 1994).

The idea that insulin stimulates ovarian androgen production by directly activating its own receptor is supported by the report of Willis and Franks (1995) that steroidgenic effects of insulin are mediated by insulin receptors as such and not by the IGF-1 receptor in primary cultures of human ovarian granulose cells. Furthermore, using human thecal cells, evidence was provided that the inositolglycan system serves as the signal transduction system for insulin stimulation of testosterone production in human ovarian thecal cells (Nestler *et al.*, 1997).

2.7.1.2 Indirect effects of insulin on androgen production

There is evidence to prove that insulin can increase LH secretion in some anovulatory, overweight women (Nestler *et al.*, 1997). Furthermore, PCOS is often characterised by abnormalities in LH secretion by the pituitary. Some studies have reported that LH pulse frequency is increased in PCOS (Burger *et al.*, 1985; Waldtreicher *et al.*, 1988; Imse *et al.*, 1992; Berga *et al.*, 1993), whereas other studies have reported no difference in LH pulse frequency between PCOS women and eumenorrhoeic women (Kazer *et al.*, 1987; Couzinet *et al.*, 1989). In general, however LH pulse amplitude appears to be increased in women with PCOS compared to a healthy age-and weight-matched control women (Berga *et al.*, 1993). Some defects in LH dynamics may be caused or aggrevated by hyperinsulinemia (Nestler, 1997).

Insulin receptors have been identified in the human pituitary gland (Unger *et al.*, 1991), and insulin has been shown to modulate anterior pituitary function *in vitro* (Yamashita & Melmed, 1986). In fact, insulin has been shown to specifically augment pituitary release of gonadotrophins *in vitro* (Adashi *et al.*, 1981). Hence, a potential mechanism whereby insulin could enchance ovarian androgen production would be by altering LH release by the pituitary. Theoretically, insulin-induced increases in either LH pulse frequency or amplitude might result in enchanced ovarian androgen production (Nestler, 1997). Insulin-enhanced LH release is supported by a finding that reducing circulating insulin with metformin leads to a decrease in basal and GnRH-stimulated LH release (Nestler and Jakubowicz, 1996).

2.7.1.3 Insulin and SHBG

Insulin influences the clinical androgenic state not only directly by affecting the metabolism of ovarian androgens, but also indirectly by regulating circulating concentrations of SHBG. Sex hormone-binding globulin binds testosterone with high affinity. It is commonly held that the unbound fraction of testosterone, and not the SHBG-bound fraction that is bio-available to tissue. Regulation of circulating SHBG by insulin constitutes an important additional mechanism by whereby insulin promotes hyperandrogenism. By reducing SHBG, insulin increases the delivery of testosterone to tissues because more testosterone is bio-available (Nestler, 1997).

To determine whether insulin can directly influence SHBG metabolism *in vivo,* the effect of insulin suppression by diazoxide on serum SHBG concentrations was examined under conditions where serum androgen and estrogen concentrations remained unchanged (Nestler *et al.*, 1991). Ovarian steroidgenesis in six obese women with PCOS was suppressed for 2 months by the administration of a long-acting GnRH agonist. Despite substantial reductions in both serum androgens snd estrogens (serum testosterone concentrations fell by 82%), serum SHBG concentrations did not change. In contrast, when diazoxide was administered for ten days to inhibit insulin release (while continuing GnRH treatment), serum SHBG concentrations rose significantly. Because ovarian steroidgenesis was suppressed in these women, diazoxide treatment did not alter after serum androgen or estrogen concentrations. Diazoxide does not alter serum SHBG values of healthy non-obese women with normal concentrations of circulating insulin (Nestler *et al.*, 1990).

Results of *in vivo* studies suggest that insulin regulates SHBG not only in obese women with PCOS but also in normal men and women (Peiris *et al.*, 1993; Preziosi *et al.*, 1993; Strain *et al.*, 1994). The results of these studies suggest that the regulation of SHBG metabolism by insulin may be a generalised physiological phenomenon, and that SHBG may serve as a biological marker for hyperinsulinemic insulin resistance in humans (Nestler, 1993).

Independently of any effect on sex steroid, increased insulin will inhibit the hepatic synthesis of SHBG (Nestler *et al.*, 1991). *In vitro* studies indicate that both insulin and IGF-1 directly inhibit SHBG secretion by human hepatoma cells (Plymate *et al.*, 1988; Singh *et al.*, 1990). This is now known to be the mechanism for the inverse relationship between body weight and the circulating levels of SHBG. Because SHBG

is regulated by insulin, decreased SHBG levels in women represent an independent risk factor for type 2 diabetes mellitus, regardless of body weight and fat distribution (Haffner *et al.*, 1993).

2.7.1.4 Insulin and follicular development

Insulin may indirectly disrupt normal folliculogenesis and the orderly flow of ovulation by increasing intra-ovarian androgens or perturbing gonadotropin release. Notably, insulin may participate in the development of PCOS not only by altering the androgen milieu or indirectly influencing ovulation, but also by directly affecting follicular development. In other words, insulin can (i) act as a mitogenic factor (ii) stimulate tissue production of other growth factors such as IGF-1 and IGF-II and, on occasion, (iii) potentiate the effects of growth factors. In any one of these ways, insulin could stimulate ovarian folliculogenesis and, ultimately, the development of multiple ovarian cysts (Ricardo *et al.*, 1997).

2.8 The clinical consequences of persistent anovulation

There are potentially severe consequences of the state of hormone secretion. Besides the problems of bleeding, amenorrhoea, hirsutism, acne and infertility, there is the increased risk of cancer of the endometrium and perhaps cancer of the breast (Coulam *et al.*, 1982; Coulam *et al.*, 1983; Ron *et al.*, 1987; Escobedo *et al.*, 1991). The risk of endometrial cancer is increased threefold, whereas it is reported that chronic anovulation during the reproductive years is associated with an increased risk of breast cancer in the postmenopausal years. However, the statistical significance of these observational studies on breast cancer was limited by small numbers, whereas orther studies have failed to find a link between anovulation and the increased risk of breast cancer (Gammon *et al.*, 1990; Gammon *et al.*, 1991; Anderson *et al.*, 1997).

The lipid profile in androgenized women with PCOS (who are also exposed to relatively lower estrogen levels over time) is similar to the male pattern. Higher levels of cholesterol, triglycerides, and LDL-cholesterol, and lower levels of HDL-cholesterol are observed in these women and this abnormal pattern is independent of body weight (Wild *et al.*, 1985; Wild *et al.*, 1990; Garf *et al.*, 1990; Conway *et al.*, 1992; Wild *et al.*, 1992). Although the elevated androgens associated with polycystic ovaries and anovulation offer some protection against osteoporosis, the adverse impact on the risk of cardiovascular disease is more important (Dicarlo *et al.*, 1992). In women undergoing coronary angiography, the prevalence of polycystic ovaries increases, and women with polycystic ovaries have more extensive coronary atherosclerosis (Birdshall *et al.*, 1997).

A contributory factor to the abnormal lipid pattern in anovulatory women is hyperinsulinemia (Wild *et al.*, 1991; Slowinska-Srzednicka *et al.*, 1991). Hyperandrogenic and hyperinsulinemic, anovulatory women must be cautioned regarding their increased risk of future diabetes mellitus. Not only are anovulatory, hyperinsulinemic women at greater risk of non-insulin-dependent diabetes, but the age of onset is about 30 years earlier than the general population (Dunaif, 1995; Legro *et al.*, 1999). It is not surprising that these patients are more likely to develop glucose

tolerance problems in pregnancy (Lanzone *et al.*, 1996). Patients who have experienced gestational diabetes are more likely to manifest the entire metabolic syndrome (hyperandrogenism and hyperinsulinemia) later in life (Holte *et al.*, 1998). In a long-term follow-up study, anovulatory women with polycystic ovaries had a fivefold increased risk of diabetes mellitus compared with age-matched controls groups (Dahlgren *et al.*, 1992). Speroff (1999, p510) states that it is essential to monitor glucose tolerance with periodic glucose-tolerance testing.

Hyperinsulinemia also contributes to the increased risk of cardiovascular disease both directly by means of atherogenic action and indirectly by adversely affecting the lipoprotein profile. Insulin resistance may be a more significant factor than androgens in determining the abnormal lipoprotein profile in overweight, anovulatory women (Wild *et al.*, 1992). However, recent research suggest that androgen concentrations may be the important determinant of risk factors for cardiovascular disease in obese women with hyperinsulinemia (Maturana *et al.*, 2002). It has also been suggested that increased insulin stimulation of IGF-1 could produce bone changes similar to those observed in acromegaly (Fox *et al.*, 1991). Hyperinsulinemia may be a factor contributing to the higher risk of endometrial cancer in these patients by increasing IGF-1 activity in the endometruim (Guidice *et al.*, 1993).

2.9 Perspective in the treatment of insulin resistance

Insulin sensitivity can be improved by non-pharmacological means, essentially reduction of excessive body weight, promotion of regular physical activity and modification of dietary habits, as well as, possibly the cessation of smoking and correction of subclinical magnesium deficiency. Currently available pharmacological means mainly include the biguanide compound metformin, thiazolodinedione derivates and possibly anti-obesity agents such as fluoxetine and benfluorex (Scheen, 1997).

2.9.1 Pharmacological approaches

There is a variety of pharmacological agents available to reduce insulin levels. Diazoxide and octreotide, long-acting analogue of somatostatin, both inhibit insulin secretion, but they are accompanied by a worsening glucose tolerance (Nestler *et al.*, 1989; Prelevic *et al.*, 1990). The best approach to improve peripheral insulin sensitivity, thus achieving reductions in insulin secretion and stability of glucose tolerance, is by administering of metformin or thiazolodinedione derivatives. These oral agents are used to treat diabetes mellitus and have been administered to anovulatory women with polycystic ovaries (Speroff, 1999, p.505)

2.9.1.1 Metformin and thiazolodinedione derivatives

Metformin improves insulin sensitivity, but the primary effect is a significant reduction in gluconeogenesis, thus decreasing hepatic glucose production. Metformin treatment reduces hyperinsulinemia, basal and stimulated LH levels, free testosterone concentrations, and plasminogen activator inhibitor-I (PAI-1) levels in overweight women with polycystic ovaries while a significant number of obese anovulatory women ovulate and achieve pregnancy following the administration of metformin (Velazques *et al.*, 1994; Nestler & Jakubowicz. 1996; Velazquez *et al.*, 1997, Diamanti-Kandarakis *et al.*, 1998). However, controversy, suggests that the improvement was the result of weight loss that often accompanies the use of metformin (Crave *et al.*, 1995). Recently, however, Awartani *et al.* (2002) indicates that there is little evidence to support the use of metformin to facilitate weigt loss. In a study designed to control the effect of body weight, the administration of metformin held no effects on insulin resistance in extremely overweight women with polycystic ovaries (Ehrman *et al.*, 1997). Similar findings in another well-designed study suggests that metformin again had no effect on insulin resistance when body weight remained unchanged. In this study the baseline weights and hyperinsulinemia were only modestly increased (Acbay *et al.*, 1996). Nestler and Jakubowicz. (1997) indicated that metformin reduced hyperandrogenemia in lean, anovulatory women with hyperinsulinemia, although there was no change in body weight; however, a decrease in the waist-to-hip ratio accompanied a reduction in hyperinsulinemia. However, in a recent study by Flemming *et al,* (2002) that compared the effects of metformin administration in a double blind placebo-controlled trial, E2 levels increased over the first week of treatment only in the metformin group. Results of the study indicated a reduction in weight loss in the group treated with metformin whereas subjects in the placebo group experienced an increase in weight. No change in fasting insulin and glucose concentrations or insulin response to glucose was observed after treatment in both groups. However there was an increase in HDL in the group treated with metformin. A inverse relationship between body mass and treatment efficacy were also found (Flemming *et al.*, 2002). Another recent study indicated that metformin administration reduces first trimester pregnancy loss in women with PCOS (Jakubowich *et al.*, 2002) whereas Kocak *et al.*, 2002 reports that metformin improves ovulation rates, cervical scores and pregnancy rates in clomiphene citrate-resistant women with PCOS.

Thiazolidinediones markedly improve insulin sensitivity and insulin secretion (improved peripheral glucose utilisation and B-cell function) without weight changes. Troglitazone (400mg daily) decreases hyperinsulinemia, and improve metabolic abnormalities (decreased androgens, increased SHBG, decreased PAI-1 consistent with improved fibrinolytic capacity, and decreased LH) and returns to ovulation in obese women have been reported with this agent (Dunaif *et al.*, 1996; Ehrmann *et al.*, 1997). It should, however, be mentioned that troglitazone (Rezulin ®) has been withdrawn form the market in March 2000 by the FDA due to liver injury which was in most cases reversible but in very rare cases ended in liver transplant or death (FDA, 2000). Rosiglitazone and pioglitazone are in the same group as troglitazone and are widely available for the treatment of type 2 diabetes mellitus. The FDA is monitoring

occurrences of adverse effect but regular liver enzyme function tests is recommended for patients taking these drugs (FDA 2000).

Speroff (1999, p506) states that appropriate clinical trials are required to answer questions regarding the effect of the short-term use of these drugs compared to standard methods of inducing ovulation. Other questions arising include whether the long-term use for preventive health care is cost-effective and how effective are these agents in women who are normal or who have only a slightly elevated body weight?

2.9.1.2 Anti-obesity agents

Several studies have indicated that serotoninergic anorectic agents, such as fenfluramine (Davis & Faulds, 1996) and fluoxetine (O'Kane *et al.*, 1994), improves glucose control in obese diabetic subjects independently of weight loss, which suggests a direct effect on insulin sensitivity (Scheen and Lefebvre, 1993). This has been confirmed using the classical euglycemic hyperinsulinemic clamp technique with the anorectic drug (Scheen *et al.*, 1991) or antidepressant compound fluoxetine (Potter van Loon *et al.*, 1992). Serotoninergic agents may therefore prove to be useful adjunct to diet or hypoglycaemic agents in obese Type 2 diabetic subjects (Scheen & Lefebvre, 1993). Their usefulness in non-diabetic insulin-resistant obese subjects, however, remains to be proven in further studies (Scheen, 1997).

Benfluorex, which is structurally related to fenfluramine, is a known hypolipidaemic agent with possible glucose-lowering effect. Benfluorex has been shown to improve glucose tolerance and lipid metabolism in obese type II diabetic patients by increasing sensitivity to insulin, without directly stimulating insulin secretion (Bianchi *et al.*, 1993; Reaven, 1993).

2.9.2 Cessation of cigarette smoking

Facchini *et al.* (1992) reported that insulin-mediated glucose uptake is significantly reduced in cigarette-smokers compared with appropriately matched non-smoking controls, and that the smokers were hyperinsulinemic and dislipidaemic. However the effect of cigarette-smoking on insulin appears to be rapidly reversible, over 10-12h (Nilsson *et al.*, 1995). It remains to be demonstrated that cigarette-smoking cessation increases the sensitivity to insulin (Sheen, 1997).

2.9.3 Magnesium supplementation

Several studies have suggested that decreased plasma and cellular magnesium levels may contribute to the insulin resistance of patients with type 2 diabetes and that this defect in insulin action can be partially reverted by magnesium supplements (Paolisso *et al.*, 1990; Lefebvre *et al.*, 1994). Further studies are essential to determine to what extent sub-clinical magnesium depletion contributes to abnormal glucose metabolism in diabetes and to evaluate the possible role of decreased magnesium content on impaired insulin sensitivity in some non-diabetic subjects (Sheen, 1997).

2.9.4 Promotion of physical activity

Results of several studies have shown (Sharma, 1992; Kriska & Bennett, 1992; Gudat *et al.*, 1994) that regular exercise may significantly improve insulin sensitivity and glucose tolerance. However, exercise-improved insulin sensitivity is usually of short duration (several days) or may require heavy and sustained training programmes which many patients may find difficult to accept (Lefebvre & Sheen, 1995). Gamineri *et al.* (2002), remarks that the treatment program for insulin resistant women with PCOS should include exercise.

2.9.5 Modification of dietary habits

Lefebvre *et al.* (1997) states that it is important to underline the importance of diet on hyperinsulinemia irrespective of weight loss. Slabber *et al.* (1994) compared the effects of two diets on serum insulin concentrations and weight loss over a 12-week period. The first diet was designed to evoke a low-insulin response (LID) and the second was a conventionally balanced energy restricted diet (ND). Insulin concentrations and weight loss were significantly reduced in subjects on the LID.

Pasquali *et al.* (1997) mention that it is theoretically possible that diet may even play a role in the development of the obesity-PCOS, although very few studies have addressed this issue. The authors also hypothesise that as both a high-lipid and low-fibre intake represent risk factors for the development of obesity, they believe that diet may partly favour hyperandrogenism in susceptible individuals but this merits further investigation. There are, however, data suggesting that women eating a vegetarian- and fibre-rich diet may have lowered androgen blood concentrations compared to women eating a typical Western diet (Hill *et al.*, 1980). Moreover, a very high lipid intake has been described in PCOS women by Wild *et al.* (1985).

2.9.6 Reduction of excessive body weight

Both hyperinsulinemia and hyperandrogenism can be reduced with weight loss, which is at least more than 5% of the initial weight (Kiddy *et al.*, 1989; Pasquali *et al.*, 1989; Kiddy *et al.*, 1992; Guzick *et al.*, 1994; Anderson *et al.*, 1995; Jakubowicz *et al.*, 1997). In one study, 60 of the 67 anovulatory women, who lost from 4 to 15kg, resumed ovulation (Clark *et al.*, 1998).

Weight loss should be considered the first line of therapy in the treatment of obese, infertile women (Galtier-Dereure *et al.*, 1997; Pasquali *et al.*, 1997; Speroff, 1999, p510). Several studies on the consequences of weight loss report an improvement in menstrual function, as measured by the resumption of ovulatory cycles or the incidence of pregnancy. Weight loss is also associated with a decrease in fasting testosterone and insulin levels and an increase in SHBG levels (Pasquali *et al.*, 1989; Kiddy *et al.*, 1992; Clark *et al.*, 1995; Hollman *et al.*, 1996).

The principles of weight loss on both clinical and endocrinological features in obese, infertile women include the reduction of total and particularly visceral fat, but also

improve menstrual cycles and infertility rate, reduce androgen and insulin concentrations and insulin sensitivity (Pasquali *et al.*, 1997).

A major improvement in clinical consequences can be achieved by weight loss, and it should be emphasised, that only a relatively small percentage of weight (5 – 10%) needs to be lost in order to beneficially impact upon insulin resistance and cardiovascular hemodynamic function (Muscelli *et al.*, 1997). Speroff (1999, p 510) remarks that the best therapy for hyperinsulinemic, hyperandrogenic obese anovulatory women is weight loss.

2.10 The normal balanced energy-restricted diet

Mahan and Escott-Stump (2000, p498) recommend that an energy-restricted diet should always be nutritionally adequate except for energy, which is decreased to the point where fat stores must be metabolised to meet daily energy needs.

a) Energy
The energy intake varies according to various factors including stature and level of activity. An average of 4800 kilojoules per day for women and 5900 kilojoules per day for men is recommended by Mahan and Escott-Stump (2000, p498).

b) Carbohydrate, protein and fat
The energy-restricted diet should be relatively high in carbohydrates; 50 to 55% of the total kilojoule intake, with generous protein intake; 15 to 25% of total calories, and the fat content of the diet should not exceed 30% of the total energy intake (Mahan and Escott-Stump, 2000, p498).

c) Vitamins and minerals
Robinson *et al.* (1996, p 373) recommend a multi-vitamin mineral supplement for all diets with energy content of less than 4200kJ per day.

d) Meal plans
The exchange system is a very popular and easily manipulated method for the planning a diet program tailored to suit the individual (Mahan and Escott-Stump, 2000, p 499).The food exchange list were designed for diabetic diets and offers a flexible and practical method to develop meal plans and menus for energy-restricted diets (Robinson *et al.*, 1990, p373).

2.11 The low-insulin-response diet

For the purpose of this study we used the low-insulin response, energy restricted diet that was designed by Slabber *et al.* (1994). The principles of the diet were based on the latest literature regarding the response of insulin to common components of foods and their combinations and these factors will be discussed in the following paragraphs.

2.12 The insulin response of nutrients in foods

Postprandial blood glucose responses have been the focus of much research because of their importance for glycemic control in diabetes (Holte *et al.*, 1997). It is now widely accepted that various foods containing equal amounts of carbohydrates can produce a wide range of blood glucose responses. The term glucose response (GR) refers to the area under the blood glucose response curve (AUC) following the ingestion of 50g of carbohydrates of a specific test food divided by the area under the blood glucose response curve (AUC) that is found after the ingestion of 50g of carbohydrate from a reference food, multiplied by 100 (Jenkins *et al.*, 1981). Initially glucose was used later as reference food in normal individuals while white bread later used as reference food for diabetic subjects (Voster *et al.*, 1990). The GR was quantified and the glycemic index (GI) was developed to rank foods according to the extent to which they increase blood glucose concentrations (Wolever *et al.*, 1991).

The IR refers to the area under the blood insulin response curve following the ingestion of 50g of carbohydrate of any test food or meal, divided by the area under the blood insulin response curve that is seen after the ingestion of a reference food or meal, multiplied by 100 (Voster *et al.*, 1990). Holte *et al.*, (1997) calculated an insulin score (IS) for each of the thirty-eight test foods in the study. The IS was calculated by dividing the AUC for 1000kJ test food by the AUC for 1000kJ white bread and multiplying the result by 100.

Several studies (Coulston *et al.*, 1980; 1981; Holte *et al.*, 1997) have indicated that the IR of specific foods can differ from the GR. Holte *et al.* (1997) indicate that glycemic responses accounted for only 23% of the variability in insulinemia and macronutrients (protein, fat, water, sugar and starch) for only 10% of the variability, thus indicating that further research is required in order to examine the relationship between postprandial insulinemia, food form, and various digestive factors to develop.

The available literature indicate the following factors that influence insulin responses:

- Amino acids.
- Protein alone and in combination with carbohydrates and fats/or fats.
- Fats.
- Carbohydrates, with specific regard to type and source of carbohydrate, processing of the starch, dietary fibre.
- Antinutrients.
- Table salt.
- Organic acids and organic salts.

2.12.1 Amino acids

Fajans *et al.* (1967) researched the effect of various amino acids and combinations of amino acids on insulin production and found that arginine and lysine had the most effect on insulin production whereas histidine had no effect and leucine and phenylalanine had the same IR. Recent research has indicated that leucine and phenylalanine had a significant increase in plasma IR (Van Loon *et al.*, 2000a). Van Loon *et al.* (2000b) also pointed out that plasma insulin levels positively correlated with plasma leucine, phenylalanine and tyrosine concentrations.

2.12.2 Protein ingested alone and/or in combination with carbohydrates and/ or fats

Thirty-four years ago Estrich *et al.* (1967) reported that protein induces an increase in insulin concentrations when ingested with carbohydrates. Wolever *et al.*, (1996), however stated that it is the source and amount of carbohydrates that determine the glucose and insulin response in lean, young non-diabetic subjects after various mixed meals with variable glycemic indices. Protein and fat intake, over the range tested in their study, appeared to have had a negligible effect on postprandial glucose and insulin responses.

Studies have indicated that protein stimulates insulin secretion in both normal and type 2 diabetic subjects (Simpson *et al.*, 1985; Gulliford *et al.*, 1989; Krezwoski *et al.*, 1986). Bornet *et al.*, (1987) state that protein has a synergistic effect on IR and that insulinotropic effect increases as the glycemic index of the carbohydrates increases.

A study by Westphal *et al.*, (1990) tested the effect of various breakfast combinations: 50g of protein or 50g of glucose or 10g, 30g, 50g of protein in combination with 50g of glucose, and found that only the diet containing 50g of protein in combination with 50g of glucose led to a significant increase in insulin response. Van Loon *et al,* (2000b) found that the amino acids: leucine, phenylalanine and tyrosine, had an even greater insulinotrophic effect if co-ingested with carbohydrate. Yaspelkis (1999) compared the effect of a carbohydrate- arginine supplement to a carbohydrate-only supplement and found that there were no differences in GR or IR between the two test supplements. However, the carbohydrate oxidation was significantly reduced in the carbohydrate-arginine supplement group.

Peters & Davidson (1993) tested the effect of the consumption of protein and fat with carbohydrate on GR and insulin requirements in subjects with Type 2 diabetes mellitus and their results confirm the findings of other studies namely that the co-ingestion of protein and fat with carbohydrate increases insulin secretion compared to the insulinogenic effects of these nutrients alone.

2.12.3 Fats

Fats show no effect on insulin concentration when ingested alone (Krotkiewski *et al.*, 1987; Collier *et al.*, 1988). However, if fats are ingested in combination with carbohydrate they decrease glucose levels and increase insulin levels (Collier *et al.*, 1983; 1988).

One of the reasons suggested for the increase in insulin is the increase in glucose-inhibiting peptide (GIP) observed after a meal containing fat. Krotkiewski *et al.* (1987) suggests that the decrease in rate of gastric emptying as seen after the ingestion of fats results in a longer carbohydrate digestion period as a reason for the increases in insulin levels. Recent studies, however, compared the effects of different amounts of dietary fats and type of fats on glucose and insulin levels. These are discussed in the following paragraphs. Christiansen *et al.* (1997) compared the effects of trans-mono-unsaturated fatty acids and saturated fatty acids on postprandial glycaemia and insulinemia in obese patients with Type 1 diabetes mellitus. The results of this study indicated that serum insulin and C-peptide levels were higher following the trans-monounsaturated fatty acid diet and saturated fatty acid diet than following the cis-monounsaturated fatty acid diet. Joanic *et al.*, (1997) indicated that GR and IR were significantly lower in the carbohydrate diet supplemented with poly-unsaturated fatty acids compared to the carbohydrate diet supplemented with mono-unsaturated fatty acids. Rasmussen *et al*, (1996) also found that saturated fatty acids increased IR more than mono-unsaturated fatty acids did.

Whitley *et al.* (1997) tested the metabolic responses to isoenergetic meals containing different amounts of carbohydrate and fat. The results of this study indicated that the plasma IR was reduced after the ingestion of meals containing only fat or a substantial amount of fats. Subjects tended to feel more sleepy and less awake 3 hours after ingesting a high-fat, low-carbohydrate meal.(Murphy *et al.*, 1995). No differences were found in insulin responses following meals containing 20g, 40g and 80g of fat with the same carbohydrate content (Murphy *et al.*, 1995).

A study that compared the effects of a high-complex carbohydrate, low-fat enteral formula (RCF) with a low-carbohydrate enteral formula (HCF) in patients with Type 2 diabetes mellitus found that glucose, insulin and C-peptide responses were higher in the HCF groups than in the RCF group (Sanz-Paris *et al.*, 1998). The researchers of this study suggest that the partial replacement of complex carbohydrate with monounsaturated fatty acids in enteral formulas may improve glycemic control in subjects with Type 2 diabetes mellitus. These results were confirmed by Craig *et al.*, (1998) who found that the amount of insulin administered to Type 2 diabetic subjects on a low-carbohydrate, modified fat enteral formula was consistently less than in subjects on a high-carbohydrate formula.

2.12.4 Carbohydrates

Wolever & Miller (1995) state that the classification of starch as simple or complex does not predict blood glucose and insulin responses. It is accepted that different foods containing equal amounts of carbohydrate can produce a wide range of GR (Holte *et al.*, 1997). GR and IR are determined primarily by the amount of carbohydrate and the rate of absorption (Wolever, 2000). The IR of various monosaccharides, disaccharides and polysaccharides differs to a great extent. This is discussed in the following paragraphs.

(i) Monosaccharides

Glucose has the highest IR compared to the other monosaccharides (Bantle *et al.*, 1983; Rodin *et al.*, 1988). In a study by Glass *et al.* (1989), most obese subjects showed an abnormal high IR to glucose. Gannon *et al.* (1986) found that if Type 2 diabetes mellitus subjects have fructose is ingested with glucose the IR was lower than with glucose alone. Bantle *et al.* (1983) explains that the reason for the lower GI and IR to fructose is because insulin is perhaps not essential in the initial metabolism of fructose.

(ii) Disaccharides

The IR to sucrose and lactose have been researched, but the IR to maltose is not available. Crapo *et al.*(1980) found that the IR to sucrose was significantly higher than the IR to fructose in normal subjects. In Type 2 diabetic subjects the IR to sucrose was the same as that to glucose (Gannon *et al.*, 1989).

Miller *et al.* (1995) compared the GI and insulin index (II) of foods with naturally occurring sucrose to foods with added sucrose and found that the median GI or II in foods with naturally occurring sucrose did not significantly differ from foods with added sucrose and that foods containing sucrose do not necessarily have high GI or II. Other researchers found that added sucrose to a traditional diet did not change the daily glycemic profiles or the calculated glycemic area under the curve (Marchini *et al.*, 1994). In a recent study by Fukomuri *et al*, (2000), the IR and GI of sucrose was significantly reduced if taken with the nucleosides inosine and adenosine. The authors of this study recommend that these nucleosides be used as a component in artificial sweeteners when mixed with sucrose and that they may be useful as a food additive to suppress increases in blood glucose and insulin.

Lee *et al.* (1998) tested the effects of glucose, sucrose and fructose on plasma insulin and glucose responses in normal subjects and compared the results to the IR and GR of white bread. They found that plasma insulin responses increased nearly linearly as carbohydrate intake increased from 0 – 100g, but glucose responses increased only by 68% and 38% as the carbohydrate content increased from 25 – 50g and from 50 – 100g, respectively. The GI values for glucose were significantly higher than the GI of white bread while the GI for fructose were significantly lower than those of white bread. In their study the GI values did not differ significantly from the II values.

The GR and IR to the sugar alcohols lactitol and xylitol are smaller than the changes discussed with glucose (Natah *et al.*, 1997).

(iii) Polysaccharides

(a) Starch

The IR of starch is influenced by the following factors:

- The type of starch.
- Fibre content.
- Processing and/or method of cooking as well as the rate of digestion.
- Starch nutrient interaction.
- Starch as such or in a mixed meal.

1. Nature of the starch

Starch consists of two fractions, namely amylose and amylopectin. Different starchy foods have different ratios of amylose and amylopectin. Amylose and amylopectin are glucose polymeres and the only polysaccharides that are hydrolysed by the human small intestine. The glucose molecules in amilose is linked with alpha-1,4 bonds, and each amilose chain are linked with hydrocarbon bonds making it a very stable structure. In amylopectin the glucose molecules are linked with alpha-1,4 and alpha-1,6 bonds making amylopectin more unstable in structure with less internal hydrocarbon bindings, making hydrolysis by amilase easier (Wolever, 1990).

In a five-week cross-over study Behall *et al.* (1989) found that the GI and IR were significantly lower after eating a meal rich in amylose than after a meal rich in amylopectin. Meals containing high amylose cornflour produced lower GI and IR than did meals containing ordinary corn meal (Granfeldt *et al.*, 1995). Heijnen *et al.* (1995) also found that the postprandial GR and IR can be lowered if the amylose content of starchy drinks and puddings is raised.

In a more recent study on the bioavailability of starch in bread rich in amylose, it was found that the GI of the bread was lowered if the high amylose maize starch is substituted for a part of wheat flour (Hoebler *et al.*, 1999).

2. Food processing and/or method of cooking

Gelatinisation of the starch molecules takes place during the cooking process. Gelatinisation is the swelling of a starch molecule in the presence of water and heat, thus making the starch more prone to digestive enzymes in the gut (Wolever, 1988). Callings *et al.* (1981) reported a higher GI after the ingestion of a cooked starch rather than an uncooked starch. Furthermore, potatoes and other starchy foods are not fully digested after cooling due to a retrograding of the starch molecule. The digestibility of a potato reduces from 97 – 88% after cooling (Englyst & Cummings, 1987). Voster *et al.* (1990) indicated that both cooled and reheated maize porridge had a lower GI than warm maize porridge and suggested that this phenomenon was due to the resistance of the retrograded starch molecules to gut enzymes.

Kanan *et al.* (1998) tested the GR and IR to fresh and frozen foods. They found that the GR was higher for fresh foods but that no consistent differences were observed between the IR of the fresh and frozen foods.

Modern food processing methods give rise to increased gelatinisation of the starch associated with higher GR and IR (Wolever, 1990). Wolever (1990) recommends that the processing of a starch must be of such a nature that the time needed form preparation is less but that the slow digestive properties are retained.

Larson *et al.* (2000) tested the severity of parboiling on three rice meals. The rice in meal one was non-parboiled (NB), the rice in meal two was mildly traditionally parboiled (TB), and the rice in meal 3 was severely pressure parboiled (PP). The GI for all three rice types was lower than the GI for white bread but they found that the GI of the severely parboiled rice was lowered by 30% compared to the non-parboiled rice meal. Differential scanning calorimetry showed the presence of amylose-lipid complexes in all three rice types but only the PP rice showed retrograded amylopectin. Larson *et al.* (1996) indicates that the amylose content and not the gelatinisation temperature may be useful criteria in the selection of low GI rice also after parboiling.

Granfeldt *et al.* (2000) found that minimal processing of oat and barley had a relatively minor effect on the GI and IR features compared with the more extensive commercial processing.

The physical state of a meal affects the endocrinological responses. Peracchi *et al.*, (2000) found that the IR and GR were significantly higher after the consumption of a homogenized meal than after a solid-liquid of the same foods.

3. Starch nutrient interaction

Starch molecules bind to proteins and lipids, rendering the starch less digestible and resulting in a lower GR (Wolever, T. 1990). When monoglycerides bind to amylose the hydrocarbon chain is situated in the centre of the amylose helix. The change in starch structure causes changes in physical characteristics including increased gelatinisation temperature, and reduced stickiness (Wolever *et al.*, 1990).

4. Fibre content

Previous studies have indicated that foods that containing vast amounts of viscous fibre have a lower GR and IR (Wolever, 1988). Cooked dry beans containing gel-forming fibre had a lower IR than starchy foods e.g. potatoes, rice, wholewheat bread and cornstarch (Voster *et al.*, 1987). Torsdottir *et al.*, (1990) also pointed out that bean flakes had significantly lower IR and GR than potato flakes.

Lu *et al.* (2000) recently tested the effect of arabinoxylan fibre on postprandial glucose and insulin responses in healthy subjects. Arabinoxylan fibre is a major component of dietary fibre in cereal grains. It was concluded that arabinoxylan fibre had a reduced IR and GR compared to the control meal.

A study comparing the effect of beans as a source of fibre to a low-fibre control meal found no significant difference in GR and IR but the meal containing bean fibre prolonged the effect of intestinally derived lipoproteins and augmented cholecystokinine response to the meal (Bourdon *et al.*, 2001).

Another recent study compared the postprandial IR and GR of buckwheat bread to bread baked with groats were compared. The bread baked with buckwheat had significant lower postprandial IR and GR and had the highest satiety score (Skrabanja *et al.*, 2001). Leinonen *et al.* (1999) found that whole kernel rye bread produced lower IR than normal wheat bread but there was no difference in GR between the two breads. Oat bran concentrate bread improved GR and IR in patients with type 2 diabetes mellitus compared to normal white bread (Pick *et al.*, 1998). Guevin *et al.* (1996) concluded that the proportion of soluble to insoluble fibre does not necessarily predict the effect of the fibre on GR and IR while the overall quality of the fibre appear to affect postprandial glucose and insulin metabolism in Type 2 diabetic patients.

A breakfast cereal containing guar gum reduced postprandial IR and GR compared to the same cereal that did not contained guar gum (Fairchild *et al.*, 1996). Bourdon *et al.* (1999) tested the effect of beta-glucan, a viscous fibre, on IR and GR and found that plasma insulin and glucose responses reduced significantly after the consumption of a meal enriched with beta-glucan. They suggest that this phenomenon occurs because the carbohydrates are absorbed more slowly after the ingestion of a high-fibre meal

When sucrose is administered with acarbose, the GR and IR are lower compared to the GR and IR of sucrose alone. This may be due to the delayed gastric emptying rate (Ranganath *et al.*, 1998). Results from another study indicated that the addition of sugar beet fibre to an enteral formula reduced postprandial GR and IR and could therefore be useful in therapeutic liquid and formula diets (Thorsdottir *et al.*, 1998).

2.12.5 Antinutrients

Antinutrients are food components that reduces the availability of nutrients when present in large amounts in foods. Lectine, phytate and amylase-inhibitors are antinutriens that function as enzyme inhibitors and influence metabolic responses. Antinutrients are present in large amounts in legumes (Wolever, 1990).

Heat can destroy lectines. Foods such as cooked or canned beans contain significant amounts of lectines. Lectines are amylase-inhibitors by binding to either the enzyme or the starch. Lectines can also reduce the uptake of sugars in the intestinal gland by binding to the surface cells thus blocking the uptake (Wolever, 1990).

Fitates are present in wholegrain mealies also function as amylase-inhibitors. Phytates are heat stable but also water-soluble and can leak out during the cooking prosess. Van Heerden *et al.*, (1990) indicated that South African breads are low in fitates. It is thus unlikely that the phytate content of these breads can influence the metabolic responses.

Amylase-inhibitors are present in a wide variety of foods including legumes, wheat, bananas and peanuts. Amylase-inhibitors are heat stable and disappears in cooked foods. However the amylase-inhibitor content of foods normally eaten is too low to have a significant effect (Wolever, 1983).

2.12.6 Effect of ripening

The influence of ripening on starch is not well-researched but the composition of the starch can change. The dry weight of a green banana is about 37% starch and reduces to more or less 3% when the same banana is ripe (Englyst & Cummings, 1986). Lunetta *et al.* (1995) found that there were no important differences in the GR of different fruits and that the GR positively correlated with the glucose content of the specific fruit.

2.12.7 Table Salt

Thorburn *et al.* (1986) found that the GR and IR of a meal are increased when a moderate amount of salt is added to the meal. Salt may increase the rate of absorption of glucose and/or starch. Chloride ions in salt act as amylase-activators and increase the activity of pancreatic amylase while sodium increases the absorption of glucose in the gut (Thornburn *et al.*, 1986). Another study confirmed that the addition of salt to bread and lentils increased the GI of the foods (Chippendal, 1997).

2.12.8 Organic acids and organic salts

A study by Liljeberg *et al.*, (1995) indicated that the addition of organic acids or their salts improved the nutritional quality of starch. When subjects consumed breads baked with sourdough, lactic acid or sodium propionate the IR and GR were reduced compared to the wholemeal bread taken alone. Liljeberg *et al.* (1996) ascribed this phenomenon to the delayed rate of gastric emptying observed after the consumption of sourdough breads. These results were confirmed by Liljeberg *et al.*, (1998) when it was pointed out that the addition of acetic acid in the form of vinegar reduces glycaemia and insulin demand via delayed gastric rate. The addition of lactic acid to boiled carrots improves both the GR and IR (Gustafsson *et al.*, 1994). Contrary to these findings, Ostman *et al.* (2001) found that lactic acid did not lower the GR and IR of fermented products. Despite low glycemic indices all milk products in this study had high II and the addition of yoghurt or pickled cucumbers to a breakfast with a high GI significantly lowered the postprandial GR and IR. It was concluded that the presence of organic acids might counteract the insulinotropic effect of mixed meals (Ostman *et al.*, 2001).

Holte *et al.* (1997) found that the insulin score for yoghurt was higher than that for ice cream and that the insulin scores of both yoghurt and ice cream were significantly higher than the glucose scores for the same foods.

2.13 An insulin index of foods (II)

Postprandial blood GR has been the focus of much research because of the importance of glycemic control in patients with diabetes mellitus. It is well accepted that foods containing equal amounts of carbohydrates can produce a wide range of blood glucose responses. However, the GI concept does not consider concurrent insulin responses and few studies have reported GI values with their accompanying insulin responses (Holte *et al.*, 1997)

Holte *et al.* (1997) compared the postprandial insulin responses to isoenergetic 1000-kJ portions of several common foods. Thirty-eight foods separated into six food categories including fruit, bakery products, snacks, carbohydrate rich foods, protein rich foods, and breakfast cereals were fed to groups of 11 to 13 healthy individuals (41 subjects participated in the study). The foods were served plain as a 1000-kJ portion with 220ml water. Finger-prick blood samples were taken every 15 min over 120 min. An insulin score was calculated from the area under the insulin response curve for each food using 1000-kJ portion (45.9g carbohydrate) white bread as the reference food (score 100%).

$$IS\% = \frac{\text{Area under the 120-min insulin response curve for 1000kJ test food}}{\text{Area under the 120-min insulin response curve for 1000kJ white bread}} \times 100$$

This equation is similar to that developed by Wolever and Jenkins (1986) for the calculation of the GI. A glucose score (not the same as the GI) for each food had also been calculated by using the same equation with the corresponding plasma glucose results. The results of this study indicated that there is a wide range of IR within each food group despite similar nutrient composition. The important Western staples like bread and potatoes were amongst the most insulinogenic foods. Similarly, the highly refined bakery products and snack foods induced substantially higher insulin secretions per kilojoule than did other foods. In contrast, pasta, oatmeal porridge and All Bran® cereal produced relatively low IR, despite the high carbohydrate contents. These results also indicated a strong correlation between the insulin score and the glucose score. However, some protein and fat-rich foods (eggs, beef, lentils, cheese, cake and doughnuts) induced as much insulin secretion as carbohydrate-rich foods (e.g. beef was equal to brown rice and fish was equal to grain bread). Some foods including ice cream, yoghurt, brown rice, baked beans, apples and brown pasta had disparate insulin- and glucose scores (Holte *et al.*, 1997).

The results of this study (Holte *et al.* 1997) highlighted the fact that an increase in insulin secretion does not neccesarily account for the low GR produced by low GI foods such as pasta and All Bran cereal (Wolever *et al.*, 1994). Furthermore equal-carbohydrate servings of foods do not necessarily stimulate insulin secretion to the same extent. For example, isoenergetic servings of pasta and potatoes showed a threefold times higher IR for potatoes while isoenergetic servings of yoghurt, baked beans and whole-grain bread produced disparate IS.

Multiple-regression analysis of the individual results indicated that the GR was a significant predictor of the IR but accounted for only 23% of the variability in insulinemia. The macronutrients (protein and/or fat, water, sugar and starch) were also significant predictors but accounted for only 10% of the variability of the insulin responses. The rate of starch digestion, the amount of rapidly available glucose and resistant starch, the degree of osmolality, the viscosity of the gut's contents, and the rate of gastric emptying are indicated as other important factors influencing postprandial insulin secretion (Holte *et al.*, 1997).

2.14 Summary

This chapter summarises the effects of obesity on reproduction with specific regard to the effects of insulin and hyperandrogenism. The effects of weight loss and infertility including the effects of a normal energy restricted diet and a low-insulin response energy restricted diet are also discussed.

CHAPTER 3:

METHODOLOGY

The methods and techniques used in the study will be discussed in this chapter. The specific techniques, apparatus, methods, study sample and procedures are given as well as the principles and composition of the low-insulin-response diet. The methods for statistical analysis and the limitations of the study are also discussed.

3.1 The description of variables

The variables that were measured for the purpose of the study as well as the different techniques that were used to measure the variables will be discussed in detail in the following paragraphs.

3.1.1 Independent variables:

For the purpose of this study the independent variables were defined as follows:

i. Normal energy-restricted diet (ND) refers to a balanced energy-restricted diet (4000 – 5000kJ) based on the guidelines for planning a conventional weight loss diet according to the food exchange list system. The meal plans are given in Appenidx 2

ii. Low-insulin-response, energy-restricted diet (LID) refers to a balanced energy-restricted diet (4000 – 5000kJ) as previously designed by Slabber *et al.* (1994) and further refined according to available literature on the insulin response of nutrients, foods and combination of foods (chapter 2.8.2). The food exchange lists used for the ND were adapted for the LID. The practical application of the LIR exchange lists and meal plan are shown in Appendices 1 A and 1 B.

3.1.2 Dependent variables:

The dependent variables were defined as follows:

i. Obesity is indicated by a body mass index greater than 30kg/m^2 according to the Quetelet's index (kg/m^2) (Lee and Neiman, 1993, p133).

ii. Body fat distribution is indicated by the waist-to-hip ratio (WHR) and is classified (Jung & Chong, 1991) as:

- Android obesity with a WHR equal and > 0.80.
- Genoid obesity with a WHR < 0.80.

iii. Weight reduction refers to the total weight loss over a period of 16 weeks.

iv. Insulin resistance refers to a fasting glucose-to-insulin ratio of less than 4.5 (Legro *et al.*, 1998).

v. Menstrual abnormalities refer to amenorrhoea, menstrual cycles longer that 35 days, spot bleedings during the midmenstrual cycle, difficulty in getting pregnant or conditions including polycystic ovarian syndrome and endometriosis. These factors were assessed with by means of a questionnaire (Appendix 3).

vi. Experience of the diet includes: The acceptability of the diet as experienced by the subject, the hunger sensations that were experienced as well as the willingness to follow the diet in the future. Experience of the prescribed diets was assessed by a measuring scale questionnaire (Appendix 4).

vii. Dietary compliance refers to the number of days per week (zero to seven) during which the subjects followed the relevant diet strictly and were assessed by means of a questionnaire (Appendix 4).

viii. Endocrinological parameters:

- Fasting glucose indicates the serum glucose concentrations measured after a ten- to twelve-hour fasting period. The 30-minute and 120-minute stimulated glucose concentrations indicate plasma glucose concentrations 30- and 120 minutes after the intake of 82.5g monohydrate glucose powder diluted in 300ml of water (Smith *et al.*, 1998, p153, Burtis *et al.*, 1999, p771). The normal reference values are:
  Fasting glucose: 3.6 – 5.8 mmol/l
  30-minute stimulated glucose: 3.9 – 11 mmol/l
  120-minute stimulated glucose: 3.9 – 7.7 mmol/l

- Fasting insulin refers to serum insulin concentrations measured after a ten- to twelve-hour fasting period. The 30- and 120-minute stimulated insulin levels indicates the plasma insulin concentrations, 30 and 120 minutes after the intake of the glucose solution. The normal reference values are:
  Fasting insulin: 5 – 15uU/mL
  30 minute-stimulated insulin: 49.73 uU/L (Slabber *et al.*, 1994).
  120 minute-stimulated insulin: 35.27 uU/L (Slabber *et al.*, 1994).

- Leptin refers to the fasting serum leptin value. The normal reference value for women is 7.4 ug/L.

- Follicle-stimulating hormone (FSH) refers to the fasting serum FSH levels. The normal reference range are:
  Follicular phase: 2.5 – 10.2 U/L.
  Midcycle phase: 3 – 33.4 U/L.
  Luteal phase: 1.5 – 9.1 U/L.

- Thyroxine (FT4) refers to the fasting serum FT4 levels. The normal reference values are: 9.0 – 23.2pmol/L.

- Luteinizing hormone (LH) refers to the fasting serum LH levels. The normal reference values for women are as follows:
  Follicular phase: 1.9 – 12.5 U/L.
  Mid cycle phase: 8.7 – 76 U/L.
  Luteal phase: 0.5 – 17 U/L.

- Estrogen refers to the fasting serum estrogen levels. The normal fasting values for females are:
  Day 3 of cycle: 40 – 253pmol/L.
  Day 10 of cycle: 231 – 606 pmol/L.
  Mid cycle: 536 – 1930 pmol/L.
  Day 16: 121 – 551 pmol/L.
  Day 20: 250 – 719pmol/L.
  Day 26: 132 – 488 pmol/L.

- Progesterone refers to the fasting serum progesterone levels. The normal female fasting reference values are:
  Folliculare phase: <0.48 nmol/L.
  Luteal phase: 10.6 – 81.3 nmol/L.
  Mid luteal phase: 14.12 – 89.14 nmol/L.

- Prolactin refers to the fasting serum prolactin levels. The normal reference values in women are: 0 – 396mU/L.

- Testosterone refers to fasting serum testosterone levels. Normal fasting levels in women are: 0.5 – 2.6 nmol/ L.

- Thyroid-stimulating hormone (TSH) refers to fasting serum TSH levels. The normal fasting range is: 0.35 – 5.5 mU/L.

3.2 Choice and standardisation of apparatus and techniques

All apparatus, measuring techniques and procedures used in the study were tested and standardised prior to the execution of the study to verify the validity and reliability of the data collected. Validity indicates the degree to which an apparatus measures what it is supposed to measure (Compton & Hall, 1974, p202). Reliability indicates the repeatability of results under the same conditions (Compton & Hall, 1974, p 204). More information on the apparatus and techniques used in the collection of data is discussed in the following paragraphs.

The anthropometrical measurements were performed by a registered dietician whereas the endocrinological parameters were assessed by a qualified chemical pathologist. Measurements were performed according to standardised methods and procedures. Individual records were kept for every subject and all relevant data were noted in these records. The record form for anthropometrical data is given in Appendix 10.

3.2.1 Apparatus

The following apparatus were used for the anthropometrical measurements. All apparatus used were calibrated prior to the execution of the study.

3.2.1.1 Digital electronic scales

A 770 Seca digital electronic scale (Freddy Hirsch – Bizerba, 75860) was used to measure the subjects weight to the nearest 0.1kg. This scale was used because it is very reliable and easy to transport. The scale was calibrated before and during the course of the study to ensure reliability.

3.2.1.2 Stadiometer

A stadiometer was used to determine the subjects' height to the nearest 0.1 cm. The stadiometer consists of a light metal frame mounted on a stand with a Perspex right-angle head board that can be moved up and down. The stadiometer can measure up to two meters.

3.2.1.3 Measuring tape

A fibreglass measuring tape was used to measure the waist and hip circumferences. The use of a fibreglass or fibreglass measuring tape is required because a measuring tape made of linen can stretch and give a false value (Heymsfield *et al.*, 1984, p40).

3.2.1.4 Bio-electrical impedance assessment (BIA): Bodystat

The calliper is the most widely used method for determining body composition and body fat percentage by skinfold measurements. Although it is costeffective and easily accessible for measuring individuals of normal weight, several problems are experienced for measuring obese individuals (Heymsfield *et al.*, 1984, p46).

For the aim of this study the Body Stat® 1500MDD was used to determine body fat percentage and body composition via bio-electrical impedance.

When an electrical current is passed through the body, it is opposed by the non-conductive tissue (mainly fat and cell membranes) and transmitted by electrolytes dissolved in water (largely found in fat-free tissue, although fat-free tissue contains about 14% water) (Brodie, 1988)

The Bodystat® 1500MDD works by passing a safe, battery-generated current through the body and measuring impedance at 50 kHz. The current is harmless and cannot be felt by the subject. The body's resistance to this current is measured by the instrument. The body fat, lean body mass, dry lean mass and total body water are predicted using this measurement (Bodystat® 1500MDD Users Manual).

BIA yields values for total body water that are very close to those obtained with dilution techniques (Kushner *et al.*, 1986). A weakness is that BIA assumes that the subjects are normally hydrated. Dehydration due to insufficient water intake, excessive perspiration, intense exercise, or caffeine or alcohol use will result in the overestimation of fat mass (Khaled *et al.*, 1988). To prevent dehydration subjects are advised to drink plenty of water, refrain from consuming caffeine and alcohol the day before testing, and avoid heavy exercise 12 hours before testing (Lee & Nieman, 1993, p 153).

Lukashi *et al.* (1985) performed multiple BIA measurements on 14 subjects over 5 consecutive days and found that BIA yielded a precision of less than 2% showing good reliability. When compared with estimates of body fat derived from underwater weighing, BIA provided to be as good as (if not slightly better than) skinfold measurements in predicting body fat (Segal *et al.*, 1985; Lukashi *et al.*, 1986). The validity of BIA and the precision of prediction of lean body mass by BIA was confirmed in the study by Segal *et al.* (1988). BIA has the advantage of being safe, convenient to use, portable, rapid, and non-invasive (Lee and Nieman, 1993, p153).

Eliakim *et al.*, (2000) indicated that results from BIA correlated with other anthropometric measurements.

3.2.2 Questionnaires

Two questionnaires were developed to be completed by the subjects. The aim of the questionnaires was to determining menstrual abnormalities (Appendix 3) and the subjects compliance with and experience of the diet (Appendix 4).

To assess the validity and reliability of the questionnaires they were evaluated by the principles for question formulation and questionnaire layout (Schnetler, 1989, pp40-88).

3.2.3 The determination of insulin resistance

There are several mechanisms proposed for the state of insulin-resistance: peripheral target tissue resistance, decreased hepatic clearance, or increased pancreatic sensitivity (Poretsky, 1991). The euglycemic clamp technique establishes a steady state of hyperinsulinemia with normal glucose level at which point the glucose infusion rate equals glucose utilisation. Adding insulin will measure the glucose uptake rate (the more insulin required, the greater the peripheral resistance, also referred to as a measure of insulin sensitivity). Studies using this technique indicate that hyperandrogenic women with hyperinsulinemia have peripheral insulin resistance and, in addition, a reduction in the insulin clearance rate due to decreased hepatic insulin extraction (Poretsky, 1991; O'Meara *et al.,* 1993). In individuals with normal glucose tolerance, the fasting insulin concentrations strongly correlate with insulin resistance (Laakso, 1993). The euglycemic clamp technique is however an invasive and expensive method.

A brief report by Parra *et al.* (1994) suggests that a fasting glucose-to-fasting insulin ratio (fasting G:I ratio) might be a useful measurement for predicting glucose-stimulated hyperinsulinemia in PCOS women. Furthermore, Legro *et al.* (1998) indicated that the fasting G:I ratio is a sensitive and specific marker for insulin sensitivity in PCOS. They found that the insulin sensitivity index (S_I) as determined by the frequently sampled intravenous glucose tolerance test (FSIGT), closely correlates with insulin action determined by the euglycemic glucose clamp technique in many insulin-resistant states, including PCOS. They have controlled for the effects of age, weight, and ethnicity in PCOS women with insulin resistance by using an appropriate control group. A ratio of less than 4.5 is consistent with insulin resistance (Legro *et al.,* 1998). Thus for the purpose of this study the fasting G:I ratio was used to determine insulin resistance.

3.3 Measuring techniques and procedures

All anthropometrical measurements and techniques were carried out by the researcher according to standardised methods and are discussed in detail the following paragraphs.

3.3.1 Body height

Body length or standing height was measured with the stadiometer to the nearest 0.1cm. The subject stands barefoot with minimal clothing to facilitate correct positioning of the body. The subject stands with heels together, arms to the side, legs straight, shoulders relaxed, and head in the Frankfort horizontal plane (look straight ahead). Heels, buttocks, scapulae, and back of the head are against the vertical board of the stadiometer. Just before measurement the subject inhales deeply, holds her breath and maintains an erect posture (stand up tall) while the headboard is lowered upon the highest point of the head with enough pressure to compress the hair (Lee & Nieman, 1993, p123).

3.3.2 Body weight

A digital electronic scale was used to measure weight. The subject stands still in the centre of the scale's platform without touching anything and with the body weight equally distributed on both feet. The weight is read to the nearest 100g (0.1kg).

3.3.3 Body mass index

Body mass index (BMI) is determined by the Quetelet-index:

$$\text{Quetelet index} = \frac{\text{body weight (kg)}}{\text{height (m}^2\text{)}}$$

Roche *et al.* (1981) found that the Quetelet-index is the best single indicator of total body fat in girls and adults.

3.3.4 Waist-to-hip ratio

The waist-to-hip ratio (WHR) was measured according to the method developed by Ashwell *et al.* (1982) and is calculated by dividing the waist circumference by the hip circumference. The WHR provides an index for regional body fat distribution and is a valuable guide in assessing health risk (Bray *et al.*, 1988) Recent research suggests that the waist circumference serves as a good indidator for obesity associated risk factors (Zhu *et al.*, 2002).

The subject's waist circumference is measured at the narrowest area below the rib cage and above the umbilicus as viewed form the front (Nieman, 1990, Callaway *et al.*, 1988). The subject stands erect, abdominal muscles relaxed, arms at the side, and feet together. The measurer faces the subject and places the flexible plastic measuring tape in a horizontal plane and measures the area of least circumference. If there is no

apparent area of least circumference the measurement is taken at the level of the umbilicus. Each measurement was taken at the end of a normal expiration as recommended by Callaway *et al.* (1988).

The hip circumference is the point of greatest circumference around the hips or buttocks with the subject standing (Callaway *et al.*, 1988). The measurer kneels beside the subject to see the maximum extension of the buttocks. The tape is placed in a horizontal plane around the hips at the point of greatest circumference, and the measurement should be taken with the tape in close contact with the skin but without indenting the soft tissue. The measurement was recorded to the nearest 0.1cm.

3.3.5 Fat percentage

The Bodystat ®1500 MDD was used to determine body composition and body fat percentage. The subject lies on her back with the muscles of the arms and legs relaxed. Two electrodes of the Bodystat are placed in a symmetrical position on the left hand and the other two electrodes are placed in the same position on the left foot. The calculate button on the electronic hardware unit is pressed for the safe electrical current to pass through the body. The various readings, including body fat percentage, are then calculated and illustrated by the hardware unit.

3.3.6 Endocrinological parameters

Standard laboratory techniques and apparatus used by the Department of Chemical Pathology in the routine blood sample analyses at the Universitas Hospital were used in the study. Both laboratories belong to the international quality-control-program. All blood samples were drawn by a chemical pathologist assisted by the researcher. The standardised techniques that were used in the collection of data on insulin, glucose, leptin, FT4, TSH, FSH, LH, progesterone, testosterone and prolactin are discussed in the following paragraphs.

3.3.6.1 Collection of blood samples

Subjects followed a 250g carbohydrate diet for three days prior to the day of baseline blood sample collection (Appendix 7). Venous blood (total of 6ml serum and 14ml blood) were taken after a 10- to 12-hour fasting period. Standard syringes, vacuum tubes and no. 21 needles were used. Each subject ingested 82.5g of monohydrate glucose diluted in 300ml water directly after the fasting blood samples were taken. Blood samples for insulin and glucose were taken 30- and 120 minutes after the glucose-dilution was drunk. After the blood was drawn the test tubes and were slowly rotated (three times). Insulin samples were kept on ice while the other samples were kept at room temperature. The blood samples were then taken to the laboratories for analysis. The serum for blood sample analysis that was not tested on a daily basis was treated according to standardised methods and frozen at –20°C until the correct amount of samples were available for testing. These blood samples were kept in the freezer rooms at the Department of Chemical Pathology at temperatures ranging from -18°C to 30°C.

3.3.6.2. Blood sample analyses

Glucose:

Glucose determinations were done on the Beckman-Coulter Syncron CX7 analyser. This system determines glucose concentration by means of an oxygen rate method using a glucose oxygen electrode.

A precise volume of sample (10 microliters) is injected into a reaction cup containing a glucose oxidase solution. The ratio used is one part sample to 100 parts reagent. Electronic circuits determine the rate of oxygen consumption, which is directly proportional to the concentration of glucose in the sample.

Biochemical reaction:
Oxygen is consumed at the same rate as glucose reacts to form gluconic acid:

$$\beta\text{-D-glucose} + O_2 \xrightarrow[H_2O]{\text{Glucose Oxidase}} \text{Gluconic acid} + H_2O_2$$

Because oxygen consumption rather than peroxide formation is measured, the only requirement for peroxide is that it must be destroyed by a path not leading back to oxygen. The addition of ethanol to the reagent causes peroxide to be destroyed in the presence of catalase without yielding oxygen, according to the following reaction:

$$H_2O_2 + \text{Ethanol} \xrightarrow{\text{Catalase}} \text{Acetaldehyde} + H_2O$$

To ensure complete destruction of the peroxide, iodide and molybdate are added to the enzyme reagent, causing the following reaction:

$$H_2O_2 + 2H^+ + 2I^- \xrightarrow{\text{Molybdate}} I_2 + 2\,H_2O$$

The reaction is effective even after the catalase activity has diminished with length of storage.

Insulin:

Insulin concentrations were analysed with the LINCO Research, Inc. Human Specific radioimmunoassay for the quantitative determination of insulin. This radioimmunoassay utilises ^{125} I-labelled human Insulin and a Human insulin antiserum to determine the level of insulin in the plasma, serum and tissue.
In radioimmunoassay, a fixed concentration of labelled tracer antigen is incubated with a constant dilution of antiserum so that the concentration of antigen binding sites on the antibody is limited, for example, only 50% of the total tracer concentration is bound by antibody. If unlabelled antigen is added to this system, there is competition between labelled tracer and unlabeled antigen for the limited and constant number of binding sites on the antibody. Thus, the amount of tracer bound to antibody will decrease as the concentration of unlabelled antigen increases. This can be measured after separating anti-body-bound from free tracer and counting one or the other, or both fractions. A standards curve is set up with increasing concentrations of standard unlabelled antigen and from this curve the amount of antigen in unknown samples can be measured.

Leptin
Leptin concentrations were analysed with the LINCO Research, Inc. Human Leptin radioimmunoassay which utilises ^{125}I-labelled Human leptin and Human leptin antiserum by the antibody/ PEG technique.

Thyroid-stimulating hormone
Serum TSH concentrations were determined with the ACS: 180 automated Chemiluminescence System. The ACS 180 TSH assay is a two-site immunoassay using direct, chemiluminometric technology, which uses constant amounts of anti-bodies. The first antibody, in the lite reagent is a monoclonal mouse anti-TSH antibody labelled with acridinium ester. The second antibody, the solid phase, is a policlonal sheep anti-TSH antibody which is covalently coupled to paramagnetic particles.

FT4 (Thyroxine)
Thyroxine concentrations were analysed with the ACS: 180 FrT4 immunoassay that uses direct chemiluminescent technology. FT4 in the patient sample competes with the acridinium ester-labelled T4 in the Lite reagent for a limited amount of polyclonal rabbit anti-T4 antibody, which covalently coupled to paramagnetic particles in the solid phase.

Testosterone
Quantitative bound and unbound testosterone levels were analysed with the ACS: 180-testosterone immunoassay. Chemiluminescent technology is used. Testosterone in the patient sample competes with the acridimium ester-labelled testosterone in the Lite reagent for a limited amount of anti-testosterone antibody bound to monoclonal mouse anti-rabbit antibody, which coupled to paramagnetic particles in the solid phase. The assay uses testosterone releasing agent to release bound testosterone from endogenous binding proteins in the sample.

Prolactin
The ACS:180 prolactin assay was used to analyse serum prolactin concentrations. The ACS:180 prolactin assay is a two-site sandwich immunoassay using direct chemiluminometric technology, which uses constant amounts of antibodies. The first antibody, in the lite reagent, is a polyclonal goat anti-prolactin antibody labelled acridinium ester. The second antibody, in the solid phase, is a monoclonal mouse anti-prolactin antibody, which is covalently coupled to paramagnetic particles.

Progesterone
Serum progesterone concentrations were analysed with the ACS:180 progesterone assay, which is a immunoassay that uses direct chemiluminescent technology. Progesterone in the patient sample binds to an acridinium ester-labelled mouse monoclonal anti-progesterone antibody in the lite reagent. Unbound antibody binds to a progesterone derivate, covalently coupled to paramagnetic particles in the solid phase.

Estrogen
Estradiol concentrations were analysed with the ACS: 180 estradiol-6 II assay which is a competitive immunoassay using direct, chemiluminescent technology. The assay derives its name from the coupling of the estradiol immunogen at the specificity-enchancing sixth position, allowing for the production of a highly specific antibody. This 17 beta-estradiol-6-antibody allows the ACS:180 Estradiol-6 assay to be used across a wide range of applications. Estradiol in the patient sample competes with acridinium ester-labelled estradiol in the lite reagent for a limited rabbit-estradiol antibody. Rabbit-estradiol is captured by mouse anti-rabbit IgG, which is coupled to paramagnetic particles in the solid phase.

Luteinizing hormone (LH)
The ACS:180 LH_2 immunoassay was used to determine luteinizing concentrations in serum. The assay which uses direct chemiluminometric technology uses constant amounts of two antibodies that have specificity for the beta subunit of the LH molecule. The first antibody, in the lite reagent, is a monoclonal mouse anti-LH antibody labelled with acridinium ester. The second antibody, in the solid phase, is a monoclonal mouse anti-LH antibody which is covalently coupled to paramagnetic particles.

Follicle stimulating hormone (FSH)
The quantitative determination of serum FSH was done by the ACS:180 FSH immunoassay which consists of constant amounts of two antibodies that have specificity for intact FSH molecule. The first antibody, in the lite reagent, is a polyclonal sheep anti-FSH antibody labelled with acridinium ester and the second antibody, in the solid phase, is a monoclonal mouse anti-FSH antibody which is covalently coupled to paramagnetic particles.

3.4 Diet therapy

3.4.1 Normal balanced, energy-restricted diet

Subjects in Group B followed the normal balanced energy-restricted diet.

An individual diet was planned for each subject according to the guidelines discussed in Chapter 2. The energy content of the diet varied from 4000 to 5000 kJ per day and was based on the ideal body weight and activity level to facilitate a weight loss of one kilogram per week. The nutritional composition of the diet was as follows: carbohydrates - 50 percent of total energy, protein – 20 percent of total energy, and fats 30 percent of total energy. The food exchange lists used in energy restricted weight loss programme (Robinson *et al.*, 1990, Mahan and Escott-Stump, 2000, p499) and adapted for general use by registered dieticians in the Free State were provided to the subjects to assist in their meal-planning. Each subjects' food preferences and eating patterns were assessed with a questionnaire (Appendix 5).

3.4.2 The low-insulin-response, energy-restricted diet (LID)

The subjects on the LID also followed individual planned diets (4000 – 5000kJ, 50 % carbohydrates, 20% protein and 30% fat). The food exchange lists and meal plans used in the normal diet were based on the LID designed by Slabber *et al.* (1994) and further refined according to the most recent literature on the insulin index and factors affecting the insulin response of foods and combinations of foods as discussed in Chapter 2 and is given in Appendices 1A and 1B. The LID differed from the ND as follows:

I. Foods rich in protein were not eaten as far as possible with carbohydrate-rich foods in the same meal because literature reports that eating the above mentioned combination evokes a higher IR (chapter 2).

II. Foods known to have a high IR or high insulin index were omitted from the exchange lists.

The exchange lists were adapted for each subject as follows:

(i) Milk exchange list

Low-fat and fat-free milk exchanges are regularly used in energy-restricted diet plans. One low-fat milk exchange consists of 12 g of carbohydrates, 8 grams of proteins and 5 grams of fat, whereas a fat-free exchange consists of less than one gram of fat (Robinson *et al.,* 1990, p41). As discussed in Chapter 2, milk products are regarded as foods with a relative high IR but for the purpose of this study foods in the milk exchange list were included due to the important contribution of these foods to a balanced diet.

(ii) The vegetable exchange list

Foods in the vegetable A list consist of negligible amounts of protein, carbohydrate, fat and energy. Thus, examining the macronutrient composition of foods in the vegetable A exchange list, it was assumed that these foods will have a minimal IR and for the purpose of this study these foods could be eaten with any other food in any meal.

Vegetables on the vegetable B exchange list consist of 7g of carbohydrates and 2g of protein (Robinson *et al.,* 1990, p41). There is no available research on the IR of vegetables and for the aim of this study vegetable B exchanges were included in the main meal of the day and could be eaten with protein exchanges.

(iii) Fruit exchange list

The GI of some fruits including apples, bananas, cherries, grapefruit, grapes, oranges, peaches, apricots and prunes were determined in normal subjects with type 2 diabetes mellitus (Jenkins *et al.,*1981a; 1984; Hoover-Plow *et al.,* 1987). These fruits showed an average GI of 50 +/- 5 (mean +- standard deviation) measured against a GI for glucose of 100. Holte *et al. (*1997) determined an insulin score for certain fruits, including apples, oranges, bananas and grapes. The group mean insulin score was 71 +/- 3 compared to the mean glucose score of 61 +/- 5.

One fruit exchange contains 15g of carbohydrates (Robinson *et al.,* 1990, p 41). Lunetta *et al.,* (1995) indicated that the GI of fruits positively correlated with the glucose content of the fruit. For the purpose of this study all fruits that are relatively high in glucose were excluded from the fruit exchange list. In the LID diet fruits could only be eaten as such or in combination with a starch exchange.

(iv) The starch exchange list

One portion of food in the starch exchange list consists of 15g of carbohydrate, 2g of protein and 0 – 3g of fat (Robinson *et al.*, 1990, p40). The factors affecting the IR of starch foods, as discussed in chapter 2 were used as guidelines to develop the starch exchange list. Foods with a relatively high IR including white bread, potatoes, puffed breakfast cereals, white rice and corn flakes were excluded from the adapted exchange list (Wolever, 1990; Holte *et al.*, 1997). According to the insulin score of foods as determined by Holte *et al.*, 1997 foods with a low and medium insulin score e.g. wholegrain and seedloaf bread, All Bran Flakes®, Muesli, Special K®, white and brown pasta, grain bread, brown rice, lentils and popcorn were included in the exchange list.

Foods rich in protein e.g. foods on the meat exchange list and foods on the milk exchange list were as far as possible not combined in the same meal with foods on the starch exchange list due to the effects on insulin secretion discussed in chapter 2.

(v) Meat exchanges

One meat exchange consists of 7g of protein and 3 to 5 g of fat (Robinson *et al.*, 1990, p40). As discussed in Chapter 2, the IR of protein foods as such is minimal if the protein is not consumed with a carbohydrate-rich food in the same meal. Subjects on both diets were, however, trained to read food labels to ensure that they include lean meat products.

(vi) The fat exchange list

Only low-fat and fat-free products were included in the exchange lists of both the LID and the ND. Mahan and Escott-Stump (2000, p498) recommend that the fat content of the diet should not exceed 30% of the total energy intake. One fat exchange consist of 5g of fat (Robinson *et al.*, 1990, p41). All subjects were trained to evaluate the fat content of foods by reading the food labels to make sure that the foods they chose were low-fat or fat-free thus reducing the total fat content of their diet.

(vii) Summary of the main principles of the LID diet

The main principles of the LID were that:

- Only foods with a known low and medium insulin score or low and medium insulin response were included on the exchange list, except foods in the milk group.

- Foods rich in protein were as far as possible not consumed in the same meal with foods rich in carbohydrates e.g.
    - Meat and starch exchanges.
    - Fruit and meat exchanges.

3.5 The sample

3.5.1 Sample selection

Subjects were recruited by means of an advertisement (Appendix 6) in the local media. A Questionnaires (Appendix 8) enabled the researcher to do the initial sifting by telephone.

3.5.2 The sample size

The Department of Bio-Statistics recommended a total sample size of 60 candidates.

3.5.3 Inclusion criteria

The following inclusion criteria had to be met for participation in the study:

- Premenopausal.
- Between the ages of 18 – 40 years.
- BMI > 30kg/m^2.
- No systemic contraceptives taken for a minimum period of 6 months.

3.5.4 Exclusion criteria

Subjects with the following attributes were excluded from the study:

- Fasting blood glucose levels >6.1 or < 3.3 mmol/L, or >11.1 and > 7.0mmol/L, respectively 30 and 120 min after oral glucose tolerance tests.
- Current smokers.
- Utilisation of chronic medication or fertility drugs.
- The presence of chronic illness, e.g cardiovascular disease.

3.6 Ethical approval

The Ethics Committee of the Faculty of Health Science of the University of the Free State gave consent to implement the study after reviewing the protocol (ETOFS NR 74/00).

3.7 Implementation of the study

3.7.1 Study design

A randomised clinical trial was performed over a period of 16 weeks. Diagram 1 illustrates the experimental study plan.

Diagram 1: The experimental study design

Advertisement in media

↓

Initial sifting (done telephonically)

↓

n = 72 candidates

↓

Information session done by the researcher (n=45)

Group presentation done by the researcher regarding:

- aim and scope of study
- what is expected from each subject
- what procedures will be performed on the subject
- informed consent

↓

Individual session (n = 42)

-weight, height and BMI

-diet history and 24-hour recall in combination with a food frequency questionnaire

↓

Subjects who gave informed consent and met the inclusion criteria

n = 37 subjects

↓

Group A / Low-insulin-response diet (19 candidates)

Group B/ Normal diet (18 candidates)

↓

3-day 250g Carbohydrate diet followed by a 10 - to 12-hour fasting period

Baseline anthropometrical measurements

Weight	Hip circumference
Height	Waist circumference
Body fat percentage (%)	Body fat mass
Lean muscle %	Lean muscle mass
Body water %	Body water mass

Baseline endocrinological parameters

Glucose (fasting, 30 min, 120 min)

Insulin (fasting, 30 min, 120 min)

Fasting	Leptin	Fasting	Estrogen
	progesterone		FT4
	testosterone		FSH
	TSH		Luteinizing hormone
	Prolactin		

Subjects start with test diets

↓

16 weeks

Weekly sessions where weight was recorded by the researcher

↓

End of trial anthropometrical measurements (same as baseline)

End of trial endocrinological measurements (same as baseline)

3.7.2 Initial consultations

After the initial sifting was done over the telephone, 45 candidates met for an information session. The researcher gave a presentation on the aim of the study as well as what would be expected from each candidate. A detailed explanation was given of all the procedures that would be performed on the subjects. After the presentation, subjects had the opportunity to ask questions about the study. The subject that volunteered to take part in the study gave informed consent (Appendix 9). Thereafter individual appointments were made in which weight and height were measured (to calculate BMI). The researcher also assessed every subject's habitual food intake by means of individual diet histories in order to calculate the individual diets.

3.7.3 Division into two groups

The Department of Biostatistics divided the 37 subjects that met the inclusion criteria into two groups by using stratified randomisation by BMI categories 30 – 35kg/m^2 and >35kg/m^2. Group A followed the LID and consisted of 19 subjects and Group B followed the ND while consisting of 18 subjects.

3.7.4 Endocrinological and anthropometrical assessments session

Blood samples were collected after the subjects followed a 3-day 250g carbohydrate diet ended by a 10- to 12-hour fasting period. All blood samples were analysed by a chemical pathologist at baseline and at the end of the 16-week trial as discussed earlier in 3.3.6. Anthropometrical measurements were taken at baseline and at the end of the trial by the researcher. However, each subject's weight was recorded weekly by the researcher in order to monitor the subjects and to keep them motivated.

3.7.5 Weekly weighing sessions

During the first individual session the researcher explained to each subject the utility of the meal plan and exchange lists as well as the recording of food intake on the food-intake record form (Appendix 11). During the following sessions the researcher ensured that subjects understood all the aspects of their individual diets and recording methods. The record form for the previous week was discussed and problem areas identified. All relevant questions were answered to ensure that the subjects fully understood the use of their diet plan, and to keep the subjects motivated to follow the prescribed diet.

3.7.6 Course of the study

The subjects started with their diets following the baseline measurement session. All subjects had to maintain their habitual exercise and/or activity patterns. During the weekly weighing sessions the researcher collected the food record forms and discussed dietary issues. Subjects who missed a weekly weighing session were motivated telephonically to attend the following session.

3.8 Limitations regarding the study

3.8.1 The diagnosis of insulin resistance and anovulation

Diagnosed insulin-resistance was an inclusion criteria in the initial study design. However due to the financial implication regarding the screening test for insulin-resistance we only diagnosed insulin resistance at baseline in subjects who met the other inclusion criteria and whom had agreed to take part in the study.

We also did not diagnose anovulation as planned initially. A great number of subjects had a very irregular cycle or amenorrhoea which made testing for anovulation difficult without a clinical examination. This had certain financial and practical implications. We did however, include subjects with menstrual cycle abnormalities.

3.8.2 Drop outs

Despite the subject's commitment to complete the study there were several drop outs in both groups. Group A had a total of six drop outs and there were ten dropouts in Group B. The reasons for each drop out are discussed in Chapter 4.

3.8.3 Dietary compliance

Dietary compliance is not easy to ascertain when subject's included in a study are free living. Two subjects in Group A and six subjects in Group B did not follow their diets and were regarded as drop outs. Results of the diet compliance as assessed by the questionnaire discussed in chapter 3.2.2 are shown in chapter 4.

3.9 Statistical analysis of the results

The statistical analysis was performed by the Department of Biostatistics, University of the Free State. Categorical variables were summarised by frequencies and percentages, and numerical variables by means, standard deviations and medians. Changes in numerical variables from baseline were summarised by means, standard deviations and medians. The comparison of the two groups with respect to these changes were done using 95% confidence intervals for differences between means, and t-tests. Non-parametric tests were also performed, and since these gave similar results to the parametric analysis their results are not reported.

3.10 Summary

The choice of techniques, apparatus and procedures used to ensure a valid and reliable collection of data is discussed in this chapter. The standardisation of techniques was ensured by using standard apparatus and procedures and all procedures were standardised prior to the execution of the study. Data collection was performed and monitored by the researcher. Problems experienced during the execution of the study are also discussed.

CHAPTER 4:

RESULTS

4.1 Introduction

In order to compare the effects of the two test diets on endocrinological and anthropometrical parameters, measurements were taken at baseline and at the end of the 16-week trial period. The data were statistically analysed and the results of the study are presented in this chapter in the following order:

- Subject's characteristics at baseline.
- Drop outs that occurred and reasons for drop outs.
- Menstrual cycle abnormalities within each group.
- Anthropometrical and endocrinological characteristics of the subjects in Group A (LID) and Group B (ND) who completed the study.
- Description of the insulin-resistant subjects in Group A (LID) and Group B (ND).
- Comparison between the effects of the two diets on endocrinological parameters in the insulin-resistant subjects.
- Compliance with and experience of the two test diets.

4.2. Subjects' characteristics at baseline

4.2.1. Anthropometrical measurements

Table 1 illustrates the mean, standard deviation (SD) as well as median of age and the anthropometrical measurements of the two groups at baseline.

Table 1: Age and anthropometrical data of Group A (LID) and Group B (ND) at baseline

	Group A (LID) n = 19			Group B (ND) n= 18		
	Mean	SD	Median	Mean	SD	Median
Age:	29.89	4.26	29.0	29.56	4.36	28.0
BMI (kg/m^2)	37.22	5.62	35.42	36.09	5.65	34.86
Lean muscle %	51.4	4.2	51.1	49.68	6.71	50.95
Lean muscle mass (kg)	51.9	5.22	51.3	51.71	9.73	51.0
Body water %	35.81	2.34	35.6	34.92	4.19	35.4
Weight (kg)	102.05	15.17	95.5	99.39	18.95	95.85
Waist-to-hip ratio (WHR)	0.79	0.05	0.8	0.78	0.09	0.78
Waist circumference (cm)	103.8	12.42	102.0	101.17	16.99	102.0
Hip circumference (cm)	130.79	10.43	127.0	129.67	14.5	127.5
Body fat %	48.59	4.21	48.9	48.3	6.43	47.5
Body fat mass (kg)	49.67	11.56	46.4	49.27	15.07	44.05
Body water mass (L)	36.24	4.15	35.9	34.77	4.47	35.0

The Department of Biostatistics divided the subjects into the two groups using stratified randomisation according to BMI categories 30 – $35kg/m^2$ > $35kg/m^2$.

4.2.2 Fasting glucose, insulin and glucose to insulin ratio of Group A (LID) and Group B (ND) at baseline

Fasting glucose and insulin concentrations were analysed at baseline to determine the number of insulin-resistant subjects in each group. As discussed in Chapter 3, the fasting G:I ratio of less than 4.5 was used to define insulin resistance (Legro *et al.*, 1998). Table 2 illustrates the fasting glucose to insulin ratio of the subjects at baseline.

Table 2: The fasting insulin, glucose and glucose-to-insulin ratio of subjects at baseline

	Group A (LID) n = 19			Group B (ND) n = 18		
	Mean	SD	Median	Mean	SD	Median
Fasting insulin (μU/mL)	22.5	10.89	19.1	20.63	6.68	20.4
Fasting glucose (mg/dL)	94.35	6.54	95.5	91.28	8.11	88.3
Fasting glucose-to- insulin ratio	5.01	2.01	4.7	4.89	1.64	4.35

4.2.3 Menstrual cycle abnormalities

The menstrual cycle abnormalities were determined by means of a questionnaire which subjects completed at baseline (Appendix 3). For the purpose of this study menstrual abnormalities included amenorrhoea, irregular menstrual cycle, menstrual cycle longer than 36 days, menstruation phase shorter than three days or longer than seven days, and difficulty getting pregnant in the past and results is shown in Table 3

Table 3: Menstrual abnormalities of subjects that completed the study.

	Group A (LID) n=13		Group B (ND) n=8	
	Frequency	%	Frequency	%
Amenorrhoea	1	7.69	0	0
Irregular cycle	8	66.67	5	62.50
Menstrual cycle longer than 36 days	4	33.33	3	42.86
Menstruate shorter than 3 days	1	8.33	1	12.5
Menstruate longer than 7 days	1	8.33	1	12.5
Difficulty getting pregnant in past	5	38.46	4	50.0

Eight subjects in Group A (LID) and five subjects in Group B (ND) showed irregular cycles whereas five subjects in the LID group had difficulty getting pregnant in the past compared to four subjects in the ND group.

4.3 Drop outs

Six of the 19 subjects (32%) in Group A and ten of the 18 (56%) subjects in Group B did not complete the study. The main reasons for the drop outs in Group A and Group B were: no transport to weighing sessions, surgery, hormone treatment, unable to attend weighing sessions, relocation to another town or city and non-compliance. The results are given in Table 4.

Table 4: Reasons for drop outs during the study

	Group A (LID) n=6		Week of trial	Group B (ND) n=10		Week of trial
Reason:	**Frequency**	**%**		**Frequency**	**%**	
Started hormone treatment	1	16.67	3	1	10.0	7
No transport to weighing session	1	16.67	3	1	10.0	4
Knee operation	1	16.67	6			
Ovaria removed	1	16.67	2			
Could not attend weighing session				1	10.0	2
Relocated				1	10.0	7
Non-compliance	2	33.33	6, 8	6	60.0	5, 7, 7, 7, 6, 5

Two subjects in Group A dropped out during week 6 and week 8 due to non-compliance whereas 6 subjects in Group B dropped out between weeks 5 to 7. Thus 60% of the drop outs in Group B compared to 33% of the subjects in Group A dropped out due to non-compliance. It is interesting to note that all the drop outs occurred during the first eight weeks of the study.

4.4 The baseline anthropometrical and endocrinological characteristics of subjects that completed the study

4.4.1 Baseline anthropometrical parameters

Table 5 illustrates the mean, SD and median of the age and anthropometrical measurements taken at baseline in subjects who completed the study.

Table 5: Baseline age and anthropometrical data of subjects in Group A (LID) and Group B (ND) that completed the study

	Group A (LID) n = 13			Group B (ND) n = 8		
	Mean	**SD**	**Median**	**Mean**	**SD**	**Median**
Age	30.31	4.52	29.0	29.5	5.04	28.5
Height (m)	1.65	0.07	1.67	1.67	0.06	1.67
Weight (kg)	99.38	13.56	95.5	108.86	23.47	104.4
BMI (kg/m^2)	36.43	5.46	35.42	38.75	6.79	36.96
Body fat %	48.1	4.18	48.9	50.63	7.92	49.8
Body fat mass (kg)	47.92	10.19	46.4	57.41	18.13	50.25
Lean muscle %	51.89	4.17	51.1	46.18	7.52	48.9
Lean muscle mass (kg)	51.09	5.25	51.3	50.95	8.38	52.25
Body water %	36.04	2.3	35.9	32.48	4.22	34.1
Body water mass (L)	35.57	4.17	35.9	36.06	5.39	35.85
Waist circumference (cm)	102.92	12.74	102.0	109.63	16.81	105.0
Hip circumference (cm)	129.38	10.56	126.0	134.38	17.48	132.5
WHR:	0.79	0.06	0.8	0.82	0.06	0.81

Table 5 indicates the baseline anthropometrical parameters for the two groups. The comparison between the differences between Group A and Group B is given in Table 6.

4.4.2 Comparison between the baseline anthropometrical characteristics of subjects that completed the study.

The mean, 95% CI as well as the mean differences between the anthropometrical parameters of Group A and Group B at baseline is shown in Table 6.

Table 6: Mean differences between the anthropometrical parameters in Group A (LID) and Group B (ND) at baseline

	Group A (LID) n= 13	Group B (ND) n= 8		
	Mean (95% CI)	Mean (95% CI)	Mean differences between the two groups	95% CI
Height (m)	1.65 (1.61; 1.69)	1.67 (1.62; 1.72)	-0.02	-0.08; 0.05
Weight (kg)	99.38 (91.19; 107.58)	108.86 (89.24; 128.48)	-9.47	-26.28; 7.32
BMI (kg/m^2)	36.43 (33.13; 39.72)	38.75 (33.07; 44 41)	-2.31	-7.93; 3.30
Body fat %	48.1 (45.57; 50.62)	50.62 (44.01; 57.26)	-2.52	-8.02; 2.97
Body fat mass (kg)	47.92 (41.76; 54.08)	57.41 (42;25; 72;57)	-9.5	-22.34; 3.3
Lean muscle %	51.89 (49.37; 54.41)	46.17 (39.89; 52.46)	5.7	0.41; 11.02**
Lean muscle mass (kg)	51.09 (47.91; 54.26)	50.95 (43.94; 57.95)	0.14	-6.05; 6.32
Body water %	36.04 (34.65; 37.42)	32.47 (28.94; 36.0)	3.5	0.61; 6.52**
Body water mass (L)	35.57 (33.05; 38.08)	36.08 (31.57; 40.58)	-0.51	-4.89; 3.87
Waist circumference (cm)	102.92 (95.22; 110.62)	109.63 (95.58; 123.67)	-6.7	-20.22; 6.8
Hip circumference (cm)	129.38 (123; 135.77)	134.38 (119.76; 148.99)	-4.99	-17.71; 7.73
WHR	0.79 (0.76; 0.83)	0.82 (0.76; 0.87)	-0.02	-0.08; 0.03

** statistical difference between the groups

Table 6 indicates that subjects in Group A (LID) had a significantly higher lean muscle percentage (P > 0.08) and body water percentage (P >0.05) than subjects in Group B (ND). There were no other statistically significant differences between the two groups.

4.4.3 Baseline endocrinological parameters of subjects who completed the study

The blood parameters that were analysed included fasting and stimulated 30 and 120-minute insulin and glucose and fasting FT4, thyroid stimulating hormone, estrogen, progesterone, prolactin, testosterone, LH, FSH and leptin. The results are given in Table 7.

Table 7: Baseline endocrinological parameters

	Group A (LID) n = 13			Group B (ND) n = 8		
	Mean	**SD**	**Median**	**Mean**	**SD**	**Median**
Fasting glucose (mg/dL)	95.21	6.69	95.5	90.06	7.23	86.5
Fasting insulin (µU/mL)	23.56	12.26	19.1	22.06	6.59	21.35
Fasting glucose-to- insulin ratio	4.99	2.24	4.7	4.36	1.08	4.25
30min insulin (µU/mL)	136.21	75.45	137.8	171.61	81.31	156.95
120min insulin (µU/mL)	127.71	101.93	93.0	103.88	74.52	96.56
30min glucose (mg/dL)	7.28	1.43	7.0	8.45	1.24	8.5
120min glucose (mg/dL)	6.1	1.46	5.8	6.02	1.9	6.8
Fasting FT4 (pmol/L)	15.1	4.08	14.4	14.0	1.26	13.8
Fasting TSH (mU/L)	2.1	1.22	1.97	1.77	0.89	1.7
Fasting Estrogen (pmol/L)	338.9	192.3	297.0	400.5	325.44	302.0
Fasting LH (U/L)	8.98	5.33	8.6	8.01	4.19	7.6
Fasting FSH (U/L)	5.81	1.77	5.4	6.01	1.64	5.85
Fasting Prolactin (mU/L)	254.31	182.88	219.0	324.25	169.43	267.5
Fasting Testosterone (nmol/L)	2.03	0.91	1.96	2.0	0.76	1.88
Fasting Progesterone (nmol/L)	5.38	12.04	2.1	5.6	9.21	2.53
Fasting Leptin (ug/L)	32.48	9.39	33.9	37.79	22.16	28.55

The mean differences between the endocrinological parameters of the two groups are given in Table 8.

4.4.4 Comparison between the mean differences in baseline endocrinological parameters

Table 8 illustrates the mean differences as well as the 95% CI between the endocrinological parameters of Group A (LID) and Group B (ND) at baseline.

Table 8: Mean differences in endocrinological parameters at baseline

	Group A (LID) n = 13	Group B (ND) n = 8		
	Mean 95% CI	Mean 95% CI	Mean Differences between the groups	95% CI
Fasting glucose (mg/dL)	95.21 (91.17; 99.25)	90. 06 (84.02; 96.11)	5.1	-1.34; 11.63
Fasting insulin (μU/mL)	23. 56 (16.16; 30.97)	22.08 (16.57; 27.58)	1.4	-8.42; 11.39
Fasting glucose-to-insulin ratio	4.99 (3.64; 6.35)	4.36 (3.46; 5.26)	0.63	-1.15; 2.41
30min insulin (μU/mL)	136.21 (90.61; 181.8)	171.61 (103.63; 239.59)	-35.4	-108.4; 37.64
120min insulin (μU/mL)	127.72 (66.13; 189.32)	103.89 (41.59; 166.18)	23.83	-63.42; 111.1
30 min glucose (mg/dL)	7.28 (6.42; 8.15)	8.45 (7.41; 9.48)	-1.17	-2.44; 0.11
120min glucose (mg/dL)	6.1 (5.22; 6.97)	6.02 (4.44; 7.6)	0.075	-1.46; 1.61
Fasting FT4 (pmol/L)	15.1 (12.64; 17.56)	14.0 (12.95; 15.06)	1.1	-2.03; 4.23
Fasting TSH (mU/L)	2.1 (1.36; 2.83)	1.77 (1.02; 2.51)	0.33	-0.71; 1.37
Fasting Estrogen (pmol/L)	338.92 (222.72; 455.13)	400.5 (128.42; 672.58)	-61.58	-296.5; 173.32
Fasting LH (U/L)	8.98 (5.76; 12.21)	8.01 (4.51; 11.51)	0.97	-3.67; 5.62
Fasting FSH (U/L)	5.81 (4.74; 6.8)	6.01 (4.64; 7.34)	-0.21	-1.83; 1.42
Fasting Prolactin (mU/L)	245.31 (195.22; 295.39)	324.25 (182.6; 465.9)	-78.94	-193.8; 35.92

Table 8 (continued):

	Group A (LID) n = 13	Group B (ND) n = 8		
	Mean 95% CI	**Mean 95% CI**	**Mean difference between the groups**	**95% CI**
Fasting Testosterone (nmol/L)	2.03 (1.48; 2.58)	2.0 (1.36; 2.63)	0.03	-0.77; 0.84
Fasting Progesterone (nmol/L)	5.38 (-1.89; 12.65)	5.6 (-2.09; 13.30)	-0.22	-10.64; 10.2
Fasting Leptin (ug/L)	32.48 (26.81; 38.16)	37.79 (19.26; 56.31)	-5.30	-19.77; 9.16

The mean 30-minute stimulated insulin concentrations and fasting estrogen concentrations were notably higher in Group B while the mean 120-minute stimulated insulin concentrations were higher in Group A. These differences were, however, not statistical significant. Regarding the other endocrinological parameters there were no statistical differences between the two groups at baseline.

4.5. Effects of the two diets on the anthropometrical and endocrinological parameters

4.5.1 Anthropometrical parameters

Table 9 illustrates the mean changes within each group as well as the differences in mean changes between the two groups in anthropometrical parameters after the 16-week study period.

Table 9: Comparison of mean changes in anthropometrical parameters between Group A (LID) and Group B (ND).

	Group A (LID) n = 13	Group B (ND) n= 8		
	Mean change; (95% CI)	Mean change; (95% CI)	Difference between two dietary treatments	95% CI
Weight (kg)	-9.87 (-12.21; -7.53)*	-9.19 (-12.5; -5.88)*	-0.68	-4.35; 2.97
BMI (kg/m^2)	-3.63 (-4.49; -2.77)*	-3.38 (-4.67; -2.08)*	-0.25	-1.63; 1.13
Body fat %	-4.15 (-5.36; -2.94)*	-6.25 (-8.45; -4.05)*	2.1	-0.03; 4.22
Body fat mass (kg)	-7.75 (-9.86; -5.64)*	-11.41 (-15.12; -7.71)*	3.67	0.03; 7.3
Lean muscle %	4.15 (2.95; 5.35)*	9.45 (1.32; 17.57)*	-5.3	-11.04; 0.45
Lean muscle mass (kg)	-0.58 (-1.84; 0.69)	2.85 (-0.6; 6.3)	-3.43	-6.25; -0.6**
Body water %	2.65 (1.82; 3.48)	6.46 (1.64; 11.3)*	-3.81	-7.27; -0.36**
Body water mass (L)	-0.01 (-1.61; 1.6)	1.59 (-1.37; 4.55)	-1.6	-4.43; 1.24
Waist circumference (cm)	-8.46 (-11.0; -5.93)*	-8.75 (-11.74; -5.76)*	0.29	-3.45; 4.03
Hip circumference (cm)	-11.31 (-13.74; -8.87)*	-12.13 (-16.71; -7.54)*	0.82	-3.53; 5.16
Waist-to-hip ratio	0.004 (-0.01; 0.02)	0.01 (-0.01; 0.02)	-0.004	-0.03; 0.02

*Statistical difference within the group

**Statistical difference between the groups

The data in Table 9 indicate that weight, BMI, body fat percentage, body fat mass, waist circumference and hip circumference significantly reduced within each group. The only significant differences between the two groups following the 16-week trial were that the lean muscle mass and body water percentage were significantly lower in Group A (P 0.07) (P > 0.03).

4.5.2 Endocrinological parameters

The mean change as well as the difference in mean change within each group and between the two groups regarding the endocrinological parameters are given in Table 10
(* Statistical difference within group ** Statistical differences between groups).

Table 10: The mean changes in endocrinological parameters between Group A (LID) and Group B (ND)

	Group A (LID) n = 13	Group B (ND) N = 8		
	Mean Change (95% CI)	Mean Change (95% CI)	Difference between two dietary treatments	95% CI
Fasting glucose (mg/dL)	-2.07 (-7.00; 2.83)	-0.65 (-4.84; 3.55)	-1.42	-8.12; 5.28
Glucose 30min (mg/dL)	-0.39 (-0.96;0.17)	-1.04 (-2.1;0.03)	0.65	-0.36;1.65
Glucose 120min (mg/dL)	-0.29 (-1.12;0.53)	0.39 (-0.89;1.7)	-0.68	-2.03;0.67
Fasting insulin (μU/mL)	-2.22 (-6.99; 2.54)	-5.59* (-10.8; -0.37)	3.36	-3.52; 10.25
Insulin 30min (μU/mL)	-9.7 (-35.57;16.17)	-55.28* (-83.62;-26.93)	45.57	8.17; 82.97**
Insulin 120min (μU/mL)	-39.95 (-82.21;2.31)	-11.15 (-49.52; 27.22)	-28.8	-87.27; 29.68
Fasting Leptin (ug/L)	-10.65* (-16.73; -4.57)	-11.85* (-19.73; -3.97)	1.2	-8.04; 10.45
Fasting FT4 (pmol/L)	-0.61 (-2.89; 1.67)	0.56 (-1.35; 0.22)	-0.05	-2.91; 2.83
Fasting TSH (mU/L)	0.4 (-0.35; 1.14)	-0.01 (-0.61; 0.59)	0.4	-0.61; 1.41
Fasting Estrogen (pmol/L)	20.69 (-159.3; 200.67)	-89.88 (-227.4; 47.64)	110.57	-131; 352.1
Fasting LH (u/L)	-1.76 (-5.43; 1.91)	-4.37* (-7.95; -0.8)	2.61	-2.54; 7.76
Fasting FSH (u/L)	-0.01 (-1.57; 1.56)	-0.76 (-2.57; 1.05)	0.75	-1.54; 3.05
Fasting Prolactin (mU/L)	13.03 (-74.73; 48.68)	-29.25 (-171.4; 112.86)	16.23	-107.2; 139.68
Fasting Testosterone (nmol/L)	-0.25 (-0.64; 0.13)	-0.55* (-0.95; -0.16)	0.3	-0.25; 0.85
Fasting Progesterone (nmol/L)	2.81 (-8.78; 14.39)	-0.004 (-10.01; 10.01)	2.81	-13.07; 18.7

There was a significant reduction in 30-minute stimulated insulin level within Group B with a statistical difference of 45.57 μU/L between the two Groups ($P > 0.01$). No other significant differences occurred between the effects of the two test diets on the endocrinological parameters. It should however be noted that fasting insulin, testosterone and luteinizing hormone significantly reduced within Group B whereas fasting leptin levels decreased significantly within both Group A and Group B.

4.6 Description of the insulin-resistant subjects in the Group A (LID) and Group B (ND)

For the purpose of this study insulin resistance was defined as a fasting glucose-to-insulin ratio of less than 4.5 (Legro *et al.*, 1998). Six subjects in Group A and five subjects in Group B were insulin-resistant according to the above-mentioned criteria. Table 11 illustrates the baseline endocrinological parameters of the insulin resistant subjects in the two groups.

Table 11: Baseline data of insulin-resistant subjects in Group A (LID) and Group B (ND)

	Insulin resistant subjects in Group A (LID) n = 6			Insulin resistant subjects in Group B (ND) n = 5		
	Mean	**SD**	**Median**	**Mean**	**SD**	**Median**
Fasting glucose (mg/dL):	93.97	7.88	95.5	91.48	8.82	86.5
Fasting insulin (uU/L):	33.5	11.33	31.5	25.46	6.08	22.9
30 min glucose (mg/dL)	7.68	1.98	7.7	8.88	0.88	8.6
120 min glucose (mg/dL)	6.47	1.93	5.8	6.34	1.81	8.6
30 min insulin (uU/L)	183.81	77.42	183.8	198.8	83.35	155.4
120 min insulin (uU/L)	191.93	120.7	155.4	131.88	77.36	129.7
Glucose-to-insulin ratio	3.05	0.99	2.7	3.76	0.78	4.1
Fasting FT4 (pmol/L)	15.93	6.03	13.9	13.8	1.42	13.7
Fasting TSH (mU/L)	1.85	1.29	1.73	1.98	1.03	2.08
Fasting Estrogen (pmol/L)	240	77.48	263.5	400	365.76	262.0
Fasting LH (uU/L)	8.65	5.72	8.0	7.12	3.09	7.6
Fasting FSH (u/L)	5.43	1.28	4.95	6.16	2.15	6.5
Fasting Prolactin (mU/L)	209.67	162.72	191.5	351.2	217.79	237.0
Fasting Testosterone (nmol/L)	2.1	0.67	2.17	1.86	0.78	1.74

Table 12 gives the statistical differences between the endocrinological parameters of the insulin-resistant subjects in Group A (LID) and Group B (ND) at baseline.

Table 12: Mean differences between the endocrinological parameters of insulin resistant subjects at baseline

	Group A (LID) n= 6	Group B (ND) n= 5		
	Mean 95% CI	Mean 95% CI	Mean Difference between the groups	95% CI
Fasting glucose (mg/dL):	93.97 (85.69; 102.24)	91.48 (80.52; 102.43)	2.4	-8.9; 13.87
Fasting insulin (μU/L):	33.5 (21.16; 45.38)	25.46 (17.91; 33.0)	8.04	-4.79; 20.86
30 min glucose (mg/dL)	7.68 (5.6; 9.76)	8.88 (7.78; 9.97)	-1.19	-3.37; 0.98
120 min glucose (mg/dL)	6.46 (4.43; 8.49)	6.34 (4.08; 8.59)	0.13	-2.45; 2.7
30 min insulin (μU/L)	183.81 (102.56; 256.07)	198.8 (95.30; 302.3)	-14.98	-124.7; 94.76
120 min insulin (μU/L)	191.93 (65.26; 318.6)	131.88 (35.81; 227.94)	60.05	-82; 202.11
Glucose to insulin ratio	3.05 (2.01; 4.08)	3.76 (2.79; 4.73)	-0.71	-1.94; 0.53
Fasting FT4 (pmol/L)	15.93 (9.6; 22.27)	13.8 (12.03; 15.57)	2.1	-4.16; 8.4
Fasting TSH (mU/L)	1.85 (0.49; 3.21)	1.98 (0.71; 3.27)	-0.14	-1.76; 1.4
Fasting Estrogen (pmol/L)	240 (158.69; 321.31)	400 (54.16; 854.16)	-160	-503; 183.26
Fasting LH (uU/L)	8.65 (2.65; 14.65)	7.12 (3.29; 10.96)	1.53	-4.96; 8.02
Fasting FSH (u/L)	5.43 (4.10; 6.76)	6.16 (3.4; 8.8)	-0.72	-3.07; 1.6

Table 12 (continued):

	Group A (LID) n = 6	Group B (ND) n = 5		
	Mean 95% CI	**Mean 95% CI**	**Mean differences between the groups**	**95% CI**
Fasting Prolactin (mU/L)	209.67 (143.85; 275.48)	351.2 (80.775; 621.63)	-141.5	-350.5; 67.41
Fasting Testosterone (nmol/L)	2.1 (1.39; 2.79)	1.86 (0.88; 2.83)	0.24	-0.75; 1.22
Fasting Progesterone (nmol/L)	2.17 (1.28; 3.05)	7.38 (-7.20; 21.96)	-5.20	-15.97; 5.55
Leptin (ug/L)	31.65 (18.86; 44.43)	43 (9.07; 67.92)	-11.35	-39.23; 16.53

The mean fasting estrogen and prolactin concentrations of the insulin resistant subjects in Group B were notably higher than in Group A whereas the 120-minute insulin concentrations were higher for subjects in Group A. These differences were, however, not statistically significant.

4.7 The effect of the two diets on the endocrinological parameters in the insulin-resistant subjects after the 16-week trial

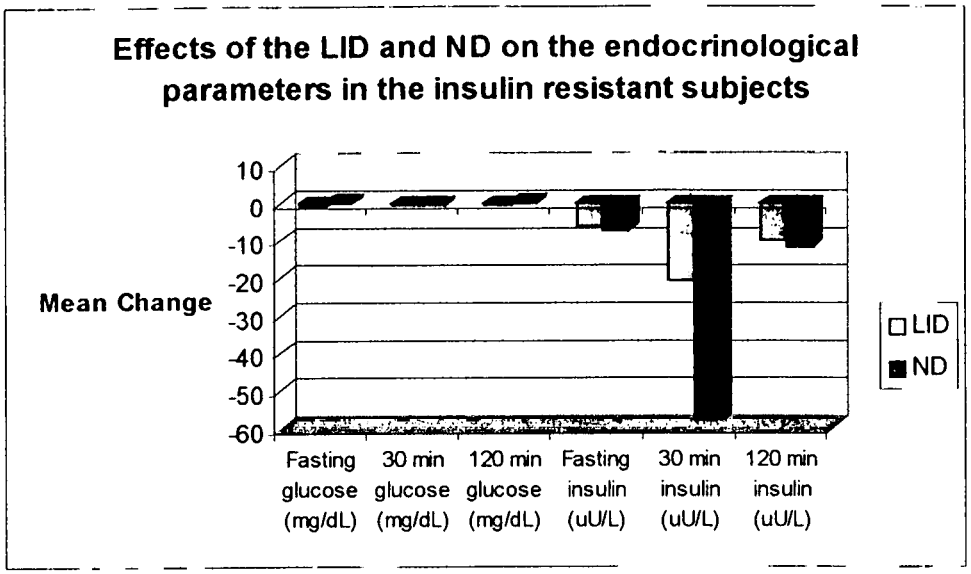
Table 13 compares the effects of the two diets on insulin and glucose concentrations in the insulin-resistant subjects in Group A and Group B.

Table 13: Comparison between the effects of the LID and ND on the endocrinological parameters in the insulin-resistant subjects in each group

	Group A (LID) n= 6	Group B (ND) n= 5		
	Mean change (95% CI)	**Mean change (95% CI)**	**Difference in mean change between two groups**	**95% CI**
Fasting glucose (mg/dL)	-0.874 (-11.99; 10.24)	0.41 (-6.75;7.57)	-1.29	-13.31; 10.74
30 min glucose (mg/dL)	-0.53 (-1.73; 0.67)	-0.68 (-1.42; 0.06)	0.15	-1.14; 1.4
120min glucose (uU/L)	-0.68 (-2.54; 1.16)	0.48 (-0.19; 1.15)	-1.16	-3.03; 0.7
Fasting insulin (uU/L)	-6.18 (16.39; 4.02)	-7.38 (15.93;; 1.17)	1.2	-10.56; 12.95
30min insulin (mg/dL)	-20.72 (-75.69; 34.62)	-57.98* (-90.66; -25.3)	37.26	-21.38; 95.9
120min insulin (uU/L)	-63.77 (-167.7; 40.15)	-13.08 (-69.68; 43.52)	-50.69	-160; 58.65
Leptin (ug/L)	-10.22 (-22.18; 1.7)	-11.68 (-26.08; 2.72)	1.46	-14.27; 17.2

*significant change within the group

There was a significant reduction in the 30-minute stimulated insulin concentrations of the insulin resistant subjects in Group B. No other statistical differences were indicated between the two groups.



4.8. 1 Dietary compliance

Compliance was measured by means of a questionnaire (Appendix 4) that was completed by subjects at the end of the study period. The results are given in Table 14.

Table 14: Dietary compliance

	Group A (LID) n=13		Group B (ND) n=8	
	Number of candidates	%	Number of candidates	%
Followed diet for 4 days per week	1	7.69	1	12.5
Followed diet for 5 days per week	7	53.85	4	50.0
Followd diet for 6 days per week	3	23.08	2	25.0
Followed diet for 7 days per week	2	15.38	1	12.5

Table 14 indicates that more subjects in the low-insulin response diet group followed the prescribed diet for five and more days per week compared to subjects on the normal diet.

4.8.2 General attitude and feelings towards the diet

Attitude towards the prescribed diet was measured by means of a questionnaire (Appendix 4). The results are given in Table 15.

Table 15: Attitude towards the diet

	Group A (LID) n= 13		Group B (ND) n= 8	
	Number of candidates	%	Number of candidates	%
Felt that diet was very acceptable	10	76.92	8	100.0
Felt that diet was moderately acceptable	3	23.08	0	0
Felt hungry at some stages	2	15.38	2	25.0
Felt hungry very few times	7	53.85	5	62.5
Did not feel hungry at all	4	30.77	1	12.5
Will follow diet in future as a maintenance diet	12	92.31	4	50.0
Will follow diet only until goal weight is reached	1	7.69	4	50.0

Only 50% of the subjects in Group B indicated that they would follow the prescribed diet in the future compared to only one of the thirteen subjects (7.69%) in Group A. It should also be noted that more than 30% of the subjects in Group A did not feel hungry on the prescribed diet compared to only 12.5% of the subjects in Group B.

4.9 Summary

This chapter presents detailed results regarding the effects of the LID and the ND on the anthropometrical and endocrinological parameters in obese women.

At the beginning of the sixteen-week trial there was a total of 19 subjects in the LID group and 18 candidates in the ND group. Six candidates in Group A (LID) and ten in Group B (ND) dropped out of the study. Only two of the six candidates who dropped out in Group A (33.33%) did not follow the diet compared to six of the ten dropouts in Group B (60%).

No statistical differences were observed between the two groups at baseline except for the lean muscle percentage and body water percentage that were significantly higher in Group A.

An analysis of the effects of the two diets on the anthropometrical parameters indicated a significant difference between the two groups regarding the lean muscle mass and the body water percentage which were significantly reduced in Group A (LID). Weight, BMI, body fat %, body fat mass, waist circumference and hip circumference were significantly reduced within each group but no statistical differences occurred between the groups.

As far as the endocrinological parameters are concerned there was a statistical difference in the 30-minute stimulated insulin concentrations between the two groups with a significant reduction in Group B (ND). Other endocrinological parameters that reduced significantly within Group B included fasting insulin, luteinizing hormone and testosterone. Fasting leptin concentrations reduced significantly within both Group A and Group B. No significant differences in the endocrinological parameters of the insulin resistant subjects were observed between the two groups. There was, however, a significant reduction in 30-minute stimulated insulin levels within Group B.

More subjects were willing to follow the low-insulin-response diet in the future rather than the normal diet whereas more subjects also indicated that they felt less hungry on the low-insulin-response diet.

CHAPTER 5:

DISCUSSION

5.1 Introduction

The results of the study are discussed to illustrate the effects of the two test diets on anthropometric and endocrinological parameters in obese women with menstrual abnormalities. Furthermore, the results of this study are compared with those of other studies.

Most data regarding the effects of weight loss on anthropometric and endocrinological parameters in obese infertile women were open studies that did not include a control group (Pasquali *et al.*, 1989; Hollman *et al.*, 1996; Butzow *et al.*, 2000), or with a control group consisting of patients who failed to complete the study programme (Kiddy *et al.*, 1992; Guzick *et al.*, 1994; Clark *et al.*, 1995; Clark *et al.*, 1998). The studies mentioned compared the effects of weight loss resulting from energy restriction in obese infertile women. Slabber *et al.* (1994) compared the effects of two test diets, namely one a diet designed to evoke a low-insulin response (LID) and a normal balanced energy-restricted diet (ND) on hyperinsulinemia in obese women. Infertility was not an inclusion criteria in the study by Slabber *et al.* (1994). In another trial by Pasquali *et al.* (1986) the effects of diet alone or combined with anti-androgenic therapy were tested on weight loss and endocrinological parameters in obese women with PCOS.

Table 1 summarises the inclusion criteria, sample size, prescribed diet and duration of the studies mentioned

Table 1: Weight loss and infertility trials:

Reference	Inclusion criteria	Sample size	Prescribed diet	Study period
Pasquali et al. (1986)	BMI > 28mg/m^2 PCOS	14 Group 1: n = 7 (diet alone) Group 2: n = 7 (diet + antiandrogenic therapy)	4200 – 5000kJ	3 months
Pasquali et al. (1989)	BMI > 26kg/m^2 Hyperandrogenic Amenorrhoeic	20	4200 – 5000kJ	8 months
Guzick et al. (1994)	Obese (130 – 200% of ideal body weight) Anovulating	12 n = 6 (weight loss, training and behaviour change programme) n = 6 (wash out period)	1680kJ - first 8 weeks and then +840kJ/ week for next 4 weeks	3 months
Slabber et al. (1994)	BMI > 30kg/m^2 Hyperinsulinemic	30 Group 1: n = 15 (LID) Group 2: n = 15 (ND)	4200 – 5000kJ	3 months
Clark et al. (1995)	BMI > 30kg/m^2 Infertile > 2 years	18 Control: n = 5 (dropouts)	Healthy eating and cooking methods, training programme and behaviour modification	6 months
Hollmann et al. (1996)	Obese Menstrual disfunction	35 (29 completed)	3000 – 6000kJ	8 moths
Clark et al. (1998)	BMI > 30kg/m^2 Infertile > 2 years	87 n = 67 completed n = 20 dropouts (control)	Healthy eating and cooking methods, training programme and behaviour modification	6 months
Huber-Buchholz *et al.*, (1999)	Infertile BMI: 27 – 45kg/m^2	18 Control group – 10 age- and weight matched women	Lifestyle changes: Healthy eating and cooking methods, and exercise programme	6 months
Butzow et al. (2000)	BMI > 30kg/m^2 Infertile	10	First 6 weeks – 1600kJ Last 4 weeks – 4200 – 5000kJ	10 weeks

5.2 The effects of the LID and ND on the anthropometric parameters

5.2.1 Weight loss

The energy restriction of both the LID and the ND were modest (4000 – 5000kJ) and were aimed at a weight loss of 0.5 – 1kg per week. Robinson *et al.* (1990, p372) indicate that a gradual weight loss of 3 – 4 kg per month is effective and does not result in severe hunger and weakness that often accompanies drastic reduction regimens. Significant weight losses were observed within both the LID and the ND groups after the four-month dietary period but there were no significant differences between the effects of the two diets. Subjects in our study, however, revealed more significant weight losses compared with other studies performed over a longer period of time. In the study by Pasquali *et al.* (1989) the 16 obese infertile women who participated in the study had a mean weight loss of 9.7 ± 3.1 kg over 8 ± 2.4 months. Clark *et al.* (1995) conducted a trial on 18 anovulatory obese women over a six-month period. The dropouts in the study were included for comparison with those who completed the programme. Over the six-month period there was a significant weight loss of 6.3 ± 4.2kg ($P<0.001$) compared to the control/dropout-group's weight loss of 1.4 ± 1.8kg. The same phenomenon was observed in another six- month study by Clark *et al.* (1998) in which the 67 infertile women in the study group lost an average of 10.2kg ± 4.3kg compared to 1.2 kg ± 3.6kg for the control/ dropout group. It should also be mentioned that the women in both trials attended weekly exercise and behaviour modifications sessions (Clark *et al.*, 1995; Clark *et al.*, 1998). Galletly *et al.* (1996) performed a six-month trial on 64 obese infertile women. They indicated a mean weight loss of 5.2 ± 5.11kg. In the study by Hollman *et al.* (1996) a weight loss of 10.2 ± 7.9kg was observed over an eight- month period. Subjects were also encouraged to increase their physical activity level (Galletly *et al.*, 1996; Hollman *et al.*, 1996)

A substantial mean weight loss from 108.0 ± 5.3 to 91.8 ± 6.0kg was observed after only 12 weeks in the trial by Guzick *et al.* (1994). However, for the first eight weeks of the study the six subjects in the treatment group followed a very low energy diet of only 1680kJ consisting of solid foods and replacement of certain meals with a liquid formula. The energy content of the diet was gradually increased to 4200kJ in the last four weeks of the trial. The subjects also participated in behaviour modification as well as exercise and training sessions. The control condition was a 12-week wash-out period and these six subjects did not receive any dietary treatment, behaviour modification or training.

It should be noted that several of the trials mentioned included a training or exercise programme or both which may have influenced on the weight loss results (Guzick *et al.*, 1994; Clark *et al.*, 1995; Clark *et al.*, 1998). It is well-documented that exercise assists in weight reduction due to an increase in energy expenditure (Robinson *et al.* 1990, p375). Aerobic exercise lowers glycogen stores and promotes the use of fat for fuel. Increased physical activity furthermore causes an energy deficit and even without diet, exercise alone can be expected to lower weight by approximately two to three kilograms depending on the intensity, duration and type of exercise (Mahan and Escott-Stump, 2000, p 504). Subjects in our trial did not follow a prescribed exercise programme and were informed to maintain their usual activity level throughout the

trial period. Different results were obtained in studies that included two test groups (Paquali *et al.* 1986; Slabber *et a.,* 1994). In the trial by Pasquali *et al.* (1989) there was no statistical difference in the mean weight loss between subjects on energy restriction alone or energy restriction combined with anti-androgenic therapy. However, results of the study by Slabber *et al. (*1994) indicated that both test diets were effective methods to reduce weight but that there was significantly more weight loss in the LID group than in the ND group (9.35 ± 2.49 and 7.14 ± 4.23, respectively).

In our trial test diets were calculated according to each subject's individual requirements. The researcher also spent a considerable amount of time at baseline with each subject to explain the use of exchange lists and individualised meal plans and exchange lists. The subjects in both groups were informed about healthy eating habits which included the correct food choices and cooking methods. During the weekly contact session the researcher monitored and motivated the subjects to adhere to their diets and subjects had the opportunity to ask questions regarding their diets. This individual approach as well as the interaction and confidence between the researcher and each subject in our trial could possibly have led to the strict adherence to diets and substantial weight loss.

5.2.2 Other anthropometric parameters

5.2.2.1 Body fat percentage and BMI

A significant reduction in body fat percentage, body fat mass and BMI were observed after both diets but no significant different effects could be illustrated. Similar results were obtained in other studies (Pasquali *et al.* 1989; Hollman *et al.* 1986; Butzow *et al.,* 2000).

5.2.2.2 Waist- and hip circumferences and WHR

The impact of an increased WHR and android body fat distribution on endocrinological parameters is well documented (Kirchner *et al.,* 1990; Pasquali *et al.,* 1991; Martin *et al.,* 1993; Sonnichen *et al.,* 1993; Hollman *et al.,* 1996; Hollman & Runnebaum, 1997). Baseline results of this trial indicated that five of the six insulin- resistant subjects in the LID group and all the insulin resistant subjects (n=5) in the ND group had a WHR > 0.8.

The mean waist and hip circumferences reduced significantly in each group. A very small improvement in the WHR was observed within each group but this was,however, not statistically significant. Other trials have reported a significant reduction in the WHR after weight loss (Pasquali *et al.,* 1989; Guzick *et al.,* 1994; Huber-Buchholz *et al.,* 1999; Butzow *et al.,* 2000).

5.3 The effects of the LID and ND on endocrinological parameters

5.3.1 Insulin concentrations

5.3.1.1 Fasting insulin

At baseline there were no statistical differences between the fasting insulin concentrations of the two groups. After the four-month dietary period the fasting insulin concentrations were significantly reduced within the ND group. This reduction was, however, not significant if compared to the LID-group. A non-statistical significant reduction was also observed in the fasting insulin concentrations in the insulin-resistant subjects of both groups. Slabber *et al.* (1994) reported that fasting insulin concentrations reduced significantly more in subjects on the LID. However, all the subjects in the study by Slabber *et al.* (1994) were hyperinsulinemic (n = 30) compared to only 10 subjects (LID: n= 6, ND: n= 5) in our trial, whereas the criteria for defining insulin-resistance in the trial by Slabber *et al.*, (1994) were different from those in this trial. In the latter study insulin resistance were defined as a fasting insulin concentration significantly higher than those of an age-matched control group consisting of women of normal weight. Piatti *et al.* (1994) tested the effects of a high-protein versus a high-carbohydrate diet in obese women. They reported a significant reduction in insulin concentrations following both diets. Other researchers also reported a significant reduction in the fasting insulin concentrations in obese subjects after diet therapy. (Pasquali *et al.*, 1989; Kiddy *et al.*, 1992; Clark *et al.*, 1995; Guzick *et al.*, 1994; Hollman *et al.*, 1996; Hubet-Buchholz *et al.*, 1999; Butzow *et al.*, 2000). However, insulin resistance was not an inclusion criterion in these trials.

5.3.1.2 Stimulated insulin concentrations

The 30-minute stimulated insulin concentrations reduced significantly in the ND group compared to those in the LID group. However, it should be noted that the mean stimulated 30-minute insulin concentrations were much higher in the ND group at baseline although this difference was not statistically significant. A significant reduction in 30-minute stimulated insulin concentrations was also observed in the insulin resistant subjects in the ND group. The stimulated 120-minute insulin concentrations decreased more in subjects on the LID than in those on the ND but this reduction was not statistically significant whereas no differences were observed in the stimulated 120-minute insulin concentrations in the insulin-resistant subjects in each group. Similar results were reported by Slabber *et al.* (1994) who found no statistical difference in the 30- and 120-minute insulin concentrations in the hyperinsulinemic subjects in both dietary groups.

5.4 The effects of the LID and ND on other endocrinological parameters

5.4.1 LH and FSH

LH concentrations decreased statistically significantly in the ND group. Butzow *et al.* (2000) who studied follicular phase nocturnal urinary LH and FSH concentrations during one menstrual cycle, also reported a significant reduction in LH concentrations within the test group. The subjects followed a very low energy diet for the first six weeks of the trial followed by a normal energy-restricted diet in the remaining four weeks. In another study by Huber-Buchholz *et al.* (1999) they promoted healthy lifestyle factors including healthy eating habits and an exercise program. After the six-month period subjects whom regained ovulation indicated a significant reduction in LH concentrations. Pasquali *et al.* (1986) only reported a reduction in LH concentrations in the subjects on the combination of anti-androgenic and diet therapy but not in the group on diet therapy alone. However, Pasquali *et al.* (1986) explained that the significant fall in gonadothrophin concentrations is an obvious consequence of anti-androgenic therapy. Other studies failed to show a reduction in LH concentrations after diet therapy (Kiddy *et al.*, 1992; Guzick *et al.*, 1994; Clark *et al.* 1995; Hollman *et al.*, 1996). A significant correlation between fasting insulin and LH concentrations were reported by Bates and Whitworth (1982), Pasquali *et al.*, (1986) and Butzow *et al.*, (2000). Our results also indicated that LH and fasting insulin concentrations decreased after the ND.

The FSH concentrations in both groups remained unchanged. Similar results were observed in most other weight loss studies (Pasquali *et al.*, 1986; Pasquali *et al.*, 1989; Guzick *et al.*, 1994; Hollman *et al.*, 1996; Butzow *et al.*, 2000) where the FSH concentrations also remained unchanged.

The LH/FSH ratio is often used as a measure to determine anovulation and fertility disorders. In a more recent trial by Butzow *et al.* (2000) the LH/FSH ratio was measured and a reduction in the ratio was observed after weight loss. Although we meaured LH and FSH concentrations we did not calculate the LH/FSH ratio or diagnose anovulation because of certain limitations discussed earlier in chapter 3.

5.4. 2 Testosterone

Testosterone concentrations reduced statistically significantly in subjects on the ND. As mentioned above, fasting insulin, and stimulated 30-min insulin also reduced significantly in the ND group. A similar reduction in testosterone and fasting insulin concentrations was reported by Guzick *et al.* (1994). Pasquali *et al.* (1986) reported a significant correlation between testosterone and fasting insulin concentrations whereas *in vivo* studies (Nestler *et al.* 1992) have demonstrated that the suppression of insulin concentrations can decrease androgen concentrations. The lower fasting and stimulated insulin concentrations observed after the ND may thus be the mechanism for the reduction in testosterone concentrations observed in the ND group. Other weight loss studies (Bates and Whitworth, 1982; Pasquali *et al.* 1989; Kiddy *et al.* 1992; Clark *et al.* 1995; Butzow *et al.*, 2000) also reported a reduction in testosterone

and fasting insulin concentrations. Huber-Buchholz *et al.*, (1999), however, did not report any differences in androgen concentrations after weight loss between women who regained ovulation and those who did not regain ovulation whereas Pasquali *et al.* (1986) showed a significant correlation between insulin and testosterone concentrations. These results suggests that testosterone might be the most sensitive reproductive hormone to a reduction in insulin concentrations.

5.4.3 Leptin

Fasting leptin concentrations reduced significantly within both test diet groups with no statistically significant differences between the two groups. Very few weight loss and fertility studies to date have evaluated the effect of weight loss on leptin concentrations. However the recent study by Butzow *et al.* (2000) reported a significant reduction in leptin concentrations. Some authors (Considine *et al.* 1995; Kennedy *et al.* 1997) reported a positive correlation between leptin, body fat percentage and BMI. The mean body fat percentage, body fat mass and BMI decreased significantly in both groups in our study and this may explain the reduction in leptin concentrations observed in both groups.

5.4.4 Other

No statistically significant changes were observed in any of the other endocrinological parameters analysed including fasting and stimulating 30- and 120-min glucose, fasting thyroid hormone (FT4), TSH, estrogen, prolactin and progesterone concentrations in and between the two groups.

5.6 The effects of the two diets on menstruation cycle and pregnancy outcomes

The majority of weight loss and infertility studies included women diagnosed with menstrual cycle disorders and infertility (Pasquali *et al.*, 1986; Pasquali *et al.*, 1989; Guzick *et al.*, 1994; Clark *et al.*, 1995; Gallety *et al.*, 1996; Hollman *et al.*, 1996; Clark *et al.*, 1998; Huber-Buchholz *et al.*, 1999; Butzow *et al.*, 2000). In our study we included women with menstrual cycle abnormalities but anovulation could not be diagnosed prior to the study due to limitations discussed earlier. The population in our study population experienced the following menstrual abnormalities: amenorrhoea, irregular cycle, history of difficulty in getting pregnant whereas one of the subjects was diagnosed with PCOS and another with hirsutism. During the course of the trial two hyperinsulinemic subjects in the LID and one hyperinsulinemic subject in the ND group reported irregular spot bleedings after a prolonged period of amenorrhoea. During week 12 of the study period one subject who indicated a weight loss 8.5kg at that stage was diagnosed as being pregnant. We could not explain the improvement in abnormalities of the menstrual cycle and fertility in these subjects because we did not evaluate the endocrinological parameters at the point where an improvement was observed.

5.7 The effects of the LID and ND on attitude towards the diet and dietary compliance

Most subjects in both groups (Group A: 53.85% and Group B: 50%) indicated that they followed their prescribed diet for at least five days per week. All the subjects in the ND group indicated that their prescribed diet was very acceptable but only half of those subjects revealed that they would follow the diet in the future. However, subjects in the LID felt less hungry and more willing to follow the diet in the future as a maintenance diet compared to subject in the LID suggesting that the LID might be more acceptable as a long-term diet programme.

5.8 Summary

After the four-month dietary period a significant weight loss was reported in both groups that compared favourably with other studies that were conducted over a longer period of time (Pasquali *et al.* 1989; Clark *et al.* 1995; Galletly *et al.* 1996; Hollman *et al.* 1996; Clark *et al.* 1998). Another study by Guzick *et al.* (1994) reported greater weight loss over a shorter period of time but the energy restriction was more severe than in our study. The mean body fat percentage and BMI also reduced significantly in both groups whereas there was no statistical significant reduction in the WHR. Fasting and 30-minute stimulated insulin, LH and testosterone concentrations reduced significantly in the ND group but there was no significant changes in the LID group. However, fasting leptin concentrations reduced significantly within both groups. No significant changes were observed in any of the other endocrinological parameters.

Individual improvements in abnormalities of the menstrual cycle occurred in both groups while one subject became pregnant after a weight loss of 8.5kg in week 12 of the study on the LID.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Significant conclusions can only be drawn after an experimental study if the methods used are valid and reliable. As discussed in chapter 3, we proved that the methods used in this study were both valid and reliable. The main problem experienced in the course of this study was the number of dropouts in each test group. However, most of the reasons for dropouts were valid and included surgery, hormone therapy, relocations and the lack of transport to the weekly weighing sessions. Non-compliance was therefore not the sole reason for dropouts in this study.

The positive effects of weight loss on fertility are well documented and weight loss is often recommended as the first step in the treatment of obese infertile women. The majority of studies regarding weight loss and fertility included dietary interventions based on energy restriction alone or combined with either drugs, exercise and/or behaviour modification. The effects of the weight loss program on endocrinological parameters varied from one study to another. The majority of the weight loss studies did not find any significant changes in LH concentrations after weight loss (Bates *et al.* 1982; Kiddy *et al.* 1992; Guzick *et al.* 1994, Clark *et al.* 1995, Hollman *et al.* 1996) whereas Butzow *et al.* (2000) indicated significant reductions in LH concentrations on a very low energy content diet. However, most of the studies reported a beneficial effect of weight loss on fertility outcomes (Pasquali *et al.* 1986; Pasquali *et al.* 1989, Guzick *et al.* 1994; Clark *et al.* 1995; Galetly *et al.* 1996; Hollman *et al.* 1996, Clark *et al.* 1998; Huber-Buchholz *et al.* 1999; Butzow *et al.* 2000). Intervention studies suggest that the effects of dietary manipulation with specific regard to insulin-lowering regimens should be investigated because of the confounding role of hyperinulinemia on hyperandrogenism and infertility.

To our knowledge, this was the first study of its kind to compare the effects of two different test diets on anthropometric and endocrinological parameters with specific regard to fertility. We evaluated the effects of a diet designed to evoke low insulin responses to a normal balanced diet whereas the energy restriction of the diets was based on the same principles. The principles of the LID were based on the available literature regarding the insulin response to common food components and their combinations.

Results from this trial indicated that both the LID and the ND had a positive effect on anthropometric parameters. A significant reduction in mean weight loss, BMI, body fat percentage, waist and hip circumferences was observed in both groups. There was more weight loss over the 16-week period compared with that in other studies conducted over a longer period of time. Fasting and 30-minute stimulated insulin, LH, and testosterone concentrations reduced significantly in the ND group whereas leptin concentrations reduced significantly in both dietary groups. An improvement in menstrual abnormalities was reported in individual cases in both groups whereas one subject on the LID became pregnant one month before the end of the trial.

Our results indicated that a normal balanced energy restricted weight loss program has beneficial effects on endocrinological parameters but that a LID did not have any advantages over a ND. There were, however, more dropouts in the ND group whereas non-compliance was the major reason for dropout. Nearly all the subjects in the LID group indicated that they would follow the test diet as a future maintenance diet suggesting that the LID was a more acceptable diet.

6.2 Recommendations

This study confirmed the positive effects of weight loss on fertility parameters as reported by other researchers. We failed to prove that dietary manipulation with specific regard to a LID had any beneficial effects over a normal weight loss diet. However, Slabber *et al.* (1994) reported an improvement in endocrinological parameters in hyperinsulinemic women after following a LID. Insulin resistance was not an inclusion criterion but both groups included hyperinsulinemic subjects. Due to certain limitations we did not diagnose anovulation or infertility but we included women with abnormalities of the menstrual cycle and a history of difficulty in getting pregnant. Most other weight loss and infertility trials however, included subjects diagnosed with anovulation and infertility. Future studies of this nature should include only hyperinsulinemic, anovulatory obese women to ensure that the subjects' characteristics in both groups are homogenized at baseline.

Life style modification with specific regard to weight loss programs is recommended as the first step in the treatment of obese infertile women (Barbieri *et al.* 1997; Galtier-Dereure *et al.*, 1997). The most recent research suggest that the treatment of hyperandrogenic, insulin resistant obese women with PCOS should include a weight loss and an exercise program with insulin-sensitizing agents e.g. metformin (Bloomgarden *et al.* 2001; Gamineri *et al.* 2002; Awartani *et al.* 2002). The effects of a weight loss program with or without antiandrogenic therapy was researched by Pasquali *et al.* (1989) but future research is needed to determine the effect of diet manipulation combined with insulin-sensitizing agents in both obese and normal weight women with hyperinsulinemic PCOS.

This trial, however, serves as a good pilot study for future avenues of investigation.

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Appendix 1A: Mealplan and example menu of a subject's in the LID group

Energy: **4300kJ**
Carbohydrate: **122g**
Protein: **58g**
Fat: **34g**

Mealplan	Example menu
Breakfast	
½ milk exchange 1 Starch exchange Artificial sweetener *Optional	½ cup of fat-free milk ½ cup of All Bran Flakes®
In between snack	
1 fruit exchange	1 medium apple
Lunch	
2 Starch exchanges Free food exchange	1 cup cooked spaghetti tomato and onion sauce (Koo®)
In between snack	
1 fruit exchange 1 starch exchange 1 Fat exchange	1 medium banana 1 slice seedloaf bread 2 teaspoons of "lite" margarine
Dinner	
4 Meat exchage 1 Vegetable B Vegetable A Salad (VegetableA) 1 Fat exchange	120g Fish/ Chicken/ Lean meat ½ cup cooked carrots (with sweetener) Cooked broccoli Mixed French salads 4 – 6 teaspoons of Low fat salad dressing
Late night snack	
1 milk exchange	150ml low fat yogurt

½ cup of fatfree/ low fat milk for coffee and tea
250ml = 1 cup
5ml = 1 teaspoon

Appendix 1B: Food exchange list for the Low Insulin Response diet:

1. How to use the meal plan and exchange system

Your prescribed diet consist of a meal plan and exchange lists. The type and amount of foods that is included in your diet was determined by your individual requirements. The exchange list allows you to select your own food choices out of the various groups.

2. The meal plan

The meal plan is used as a guideline to make your food choices. The meal plan is the program of your diet. It determines the quantity of a certain type of food that you eat at a certain time. In the meal plan food quantities is described in exchanges and can be chosen from the exchange list.

3. The Exchange lists

The exchange list means that certain foods can be exchanged for other foods on the same exchange list. Exchange list is designed to offer the dieter the opportunity to eat a variety of foods. There is seven exchange lists that you can choose from. These exchange lists include a starch- fruit, milk, meat-, vegetable A-, vegetable B and fat exchange list. Also included is a free food list.

Food on the same exchange list can be substituted with one another. All the foods in the same exchange list are equal in energy-, carbohydrate, fat and protein content and may therefor be substituted with each other. All amounts on the exchange list are expressed as one exchange. For example any a food on the starch list can be substituted with any other food on the starch exchange list. Just note that food from different exchange list can not be substituted for each other (e.g. food on the starch list can not be substituted with food on the meat exchange list).

4. Basic guidelines

4.1 Eat as prescribed on the prescribed times and try as much as possible not to skip a meal.

4.2 Only choose from the foods on you exchange list and do not include foods not on your exchange lists

4.3 The success of your diet is in the type of foods in your exchange list and the combination of foods in meals. The main principle of your diet is that foods containing mainly carbohydrates must as far as possible not be consumed with foods consisting of mainly protein. To summarise:

- Starch exchanges may as far as possible not be eaten in the same meal with meat exchanges
- Fruit exchanges may as far as possible not be eaten in the same meal with meat exchanges
- Starch and fruit exchanges may be combined in the same meal
- Meat exchanges may be combined with vegetable A and vegetable B exchanges

STARCH EXCHANGE LIST

ONE STARCH PORTION EQUALS:

15 grams carbohydrates, 3 grams protein, 0-1 grams fat and 285 kJ

BREAKFAST CEREALS	**one portion is:**
All bran flakes	125 ml(½cup)
Muesli ("Lite"without nuts)	50 ml($^1/_5$cup)
Oats	60 ml($^1/_4$ cup)
Kellogg's Special K	125ml (½ cup)
Kellogg's Raisin bran	125ml (½ cup)

BREAD: **one portion is:**

Only bread with whole grains may be eaten (the bread is usually heavy and the whole grains are visible on the surface and inside the bread)

e.g. Sasko, Whole wheat bread	1 thin slice (30g)
Hyperama, Whole wheat bread	1 thin slice (30g)
Pick & Pay, farmstyle whole wheat bread	thin slice (30g)
Woolworth's seedloaf bread	1 thin slice (30g)
Woolworth's seedbuns	½ bun (30g)
Woolworths wholewheat	1 thin slice (30g)
Pick & Pay farmstyle seedloaf bread	1 thin slice (30g)
Woolworth's pumpkernikel bread	1 thin slice (30g)
Pumkernikel bread	1 thin slice (30g)

GRAINS AND OTHER STARCHES	**one portion is:**
Pasta (spaghetti, macaroni, **not** two minute noodles)	125ml(½cup)
Rice ,long grain	80ml (1/3 cup)
e.g. Basmati rice/ Tastic Tasmati rice	
Samp	125ml (1/2 cup)
Popcorn (popped, without fat/ sugar)	2cups (500ml)

STARCHY VEGETABLES	**one portion is:**
Corn, mealies	125 ml(½cup)
Corn on cob	1 medium (150 g)
Mixed vegetables (with corn)	250ml(1cup)
Sweet potato	80 ml($^1/_3$cup)

SOUPS	
Thick homemade soup (without potato and meat)	375ml (1 ½ cup)
Cup of soup lite	1 ½ packet
Canned soup	1 ½ cup (375ml)
e.g. Woolworth's, Low fat and fat free soup in can	

DRIED BEANS, PEAS, AND LENTILS ....one portion is:	
Baked beans with tomato sauce	80 ml ($^1/_3$cup)
Beans, peas and lentils	125ml(½cup)

FRUIT EXCHANGE LIST

ONE FRUIT PORTION EQUALS:

15 grams carbohydrate and 250 kJ

FRUIT	one portion is:
Apple, with peel	1 small (120 g)
Apricots	4 whole (150g)
Banana, peeled (stiff with green/ yellow skin)	1 small (80g)
Cherries	12 (100 g)
Grapefruit	1 (230 g)
Grapefruit sections, canned (no sugar)	375ml(1½cup)
Grapes	20 (100g)
Kiwi fruit	1 large (110 g)
Nectarine	2 small (150 g)
Orange, peeled	1 medium (180g)
Peach	1 medium (80 g)
Pear	1 small (100 g)
Plums	2 large (150 g)
Tangerines	2 medium (150 g)

MILK EXCHANGE LIST

0NE MILK PORTION EQUALS:

12 grams carbohydrate, 8 grams protein, 0 grams fat and 340 kJ (Skimmed)
12 grams carbohydrate, 8 grams protein, 5 grams fat and 530 kJ (Low fat)

SKIMMED MILK	one portion is:
Milk, fat free dried powder	80 ml (1/3 cup)
Milk, skim (fat free)	250 ml (1 cup)
Yoghurt, plain, fat free	180 ml (3/4 cup)
Yoghurt, fat free & artificially sweetened (slimmers choice)	180ml (3/4 cup)

e.g. Parmalat fat free yoghurt
Ghero, fat free yoghurt
Woolworth's, fat free slimmers choice yoghurt
Pick & Pay fat free yoghurt
In shape, fat free yoghurt

LOW-FAT	one portion is:
Yoghurt, plain, low-fat	180 ml (3/4 cup)
Yoghurt, low-fat, sweetened	125 ml (½ cup)

e.g. Fruits of the forest, low fat yoghurt
Parmalat, low fat yoghurt

VEGETABLE EXCHANGE LIST

ONE VEGETABLE PORTION EQUALS:

negligible amounts of carbohydrate, protein, fat and energy (Vegetable A)
7 grams carbohydrate, 2 grams protein and 150 kJ (Vegetable B)

VEGETABLE A (can be used in unrestricted amounts, without margarine and sugar)

Artichoke	Mushrooms
Artichoke hearts	Okra
Asparagus	Pea pods
Beans (green, wax, Italian)	Radishes
Bean sprouts	Salad greens
Beetgreens	Sauerkraut
Broccoli	Spinach
Brussels sprouts	Summer squash
Cabbage	Tomato
Carrots raw	Tomatoes, canned
Cauliflower	Tomato sauce
Celery	Tomato juice
Green onions or scallions	Water chestnuts
Lettuce	Watercress

Mixed vegetables (without corn, peas or pasta)

In general, one vegetable B exchange is:

- 250 ml (1 cup) raw vegetables; or
- 125 ml (½ cup) cooked vegetables.

VEGETABLE B

Beetroot	Hubbard squash
Boerpampoen	Mixed vegetables, with corn, peas and carrots
Butternut	
Carrots, cooked	
Green peas	Onion

Vegetables on List A cooked with potato and onion

MEAT AND MEAT SUBSTITUTES LIST

0NE MEAT PORTION EQUALS:

7 grams protein, 3 grams fat and 230 kJ (lean meat)
7 grams protein, 5 grams fat and 315 kJ (meat with a medium fat content)
7 grams protein, 8 grams fat and 420 kJ (meat with a high fat content)

In general, one meat portion is:

- 30 g meat;
- 30 g fish;
- 30 g poultry;
- 30 g cheese; or
- 1 egg.

BEEF	**one portion is:**
Beef, trimmed of fat	30 g
Beef, minced	30 g

CHEESE	**one portion is:**
Cheese e.g. Cheddar, gouda	30 g
Cheese, fat-free	30 g
Cottage cheese, non-fat or low-fat	60 ml ($^1/_4$ cup)
Feta	30 g
Mozzarella	30 g
Parmesan, grated	30 ml
Ricotta	60 g (60 ml)

30g cheese = "matchbox size"

FISH	**one portion is:**
Clams, crab, lobster, scallops, shrimp	30 g
Hake, Kingklip, Snoek, Cod, flounder, haddock trout	30 g
Herring (uncreamed or smoked)	30g
Oysters	6 medium
Salmon (fresh or canned)	30 g
Sardines (canned)	2 medium
Tuna (canned in salt water)	30 g

GAME	**one portion is:**
Duck, pheasant (no skin), venison, ostrich	30 g
Rabbit	30 g

LAMB	**one portion is:**
Chop (without crumbs)	30 g
Leg, roast	30 g

PORK	**one portion is:**
Lean pork, such as fresh ham; canned, cured or boiled ham; tender loin, loin chop	30g

POULTRY	**one portion is:**
Chicken (no skin).	30 g
Turkey (no skin)	30 g
Goose (well drained of fat, no skin)	30 g

MEAT EXCHANGE LIST CONTINUES:	
VEAL	**one portion is:**
Cutlet (without crumbs)	30 g
Leg of lamb, roast	30 g

OTHER	**one portion is:**
Egg	1
Egg whites	30 g
Kidney, liver, heart	30 g

FAT EXCHANGE LIST

0NE FAT PORTION EQUALS:

5 grams fat and 190 kJ

SPREADS	**one portion is:**
Margarine, medium fat spread	10 ml (2t)
e.g. Floro light	
Margarine, low fat spread,	15 ml (3t)
e.g. Floro extra light	
Peanut butter, smooth or crunchy	10 ml (2t)
FRUIT, NUTS AND SEEDS	**one portion is:**
Avocado, medium	1/8 (30 g)
Nuts: almonds, cashews	6 nuts
mixed (50% peanuts)	6 nuts
peanuts	10 nuts
pecans	4 halves
walnuts	4 halves
Olives: ripe (black)	8 large
green stuffed	10 large
Seeds:pumpkin,sesame seeds,sunflower	15 ml
SALAD DRESSINGS AND OIL	**one portion is:**
Mayonnaise, regular	10ml (2t)
Mayonnaise, low oil	20-30 ml (4-6 t)
Oil (canola, olive, peanut)	5 ml (1t)
Oil (corn, sunflower, soybean)	5 ml (1t)
Salad dressing regular	10ml (2t)
Salad dressing, low-oil"lite"	30 ml (6t)
e.g. Knorr Light, salad dressing	
Royco Light salad dressing	

1t = 1 teaspoon (5ml)

FREE FOOD LIST

Foods with a low energy content are on this list.

Artificial sweeteners (Saccharine, cyclamate, aspartame eg.) **Sweetex, Suganon, Canderel, Hermesetas, Sweet & Low, Soet en Slank, Natreen)**
Bouillon, broth, consomme
Bovril
Coffee (black)
Diet Cold drinks **(Coke Lite, Diet Fanta, Scweppes Lite, Diet Sprite, Tab, Sweet Aid, Sweeto, Weigh Less Low Cal Fruit drink, Oros lite)**
Curry powder
Fish paste
Flavourings (paprika, garlic, celery salt, parsley, nutmeg, onion salt, cinnamon)
Garlic
Gelatine (unsweetened)
Herbs
Jelly (sugar free)
Lemon juice
Marmite
Mustard
Pickles (unsweetened)
Salsa (not more than $^{1}/_{4}$ cup)
Soda water
Soy sauce
Spices
Sugar free gum
Tea
Vinegar
Worcester sauce

Appendix 2: Mealplan and example menu of a subject's in the ND group

Energy: **4300kJ**
Carbohydrate: **122g**
Protein: **58g**
Fat: **34g**

Mealplan	Example menu
Breakfast	
½ milk exchange 1 Starch exchange Artificial sweetener *Optional	½ cup of fat-free milk ½ cup of Kellogg's Corn flakes®
In between snack	
1 fruit exchange	1 medium apple
Lunch	
2 Starch exchanges 1 Fat exchange 1 Meat exchange Free food exchange	2 slices of white bread 2 teaspoons of "lite" margarine 30g of grated cheese (matchbox size) Tomato slices
In between snack	
1 fruit exchange	1 medium banana
Dinner	
3 Meat exchange 1 Starch exchange 1 Vegetable B Vegetable A Salad (VegetableA) 1 Fat exchange	90g Fish/ Chicken/ Lean meat 1 medium potato boiled ½ cup cooked carrots (with sweetener) Cooked broccoli Mixed French salads 4 – 6 teaspoons of Low fat salad dressing
Late night snack	
1 milk exchange	150ml low fat yogurt

½ cup of fatfree/ low fat milk for coffee and tea
250ml = 1 cup
5ml = 1 teaspoon

Appendix 3: Questionnaire regarding the menstrual abnormalities

Please answer the following questions by choosing the option that most applies to you in the space provided

1. **Name**_____

2. **Age**_____

3. **Do you menstruate**

   [1] – Yes
   [2] – No

4. **If you answered yes at question 3:**

   [1] – do you have a regular cycle e.g. do you menstruate every last Monday of the month etc.
   [2] – do you have a very irregular cycle e.g. can you never predict when your menstruation must start

5. **Do you experience a very long cycle – longer than 36 days (with day one of menstruation as day one of the cycle – thus is it longer than 36 days between menstruations)**

   [1] – Yes
   [2] – No

6. **Please indicate the amount of average amount of days that you menstruate**

   [1] – 3 to 7 days
   [2] – less than 3 days
   [3] – longer than 7 days

7. **Do experience very heavy flow during menstruation**

   [1] – always
   [2] – sometimes
   [3] – never

8. **If you indicated option 1 or 2 at question 7, do you experience heavy flow only on:**

   [1] – only on certain days of menstruation
   [2] – during the whole menstruation period

9. **do you experience spot bleedings between your menstruation periods or directly after the menstruation period (brown or red discharge)**

   [1] – yes
   [2] – sometimes
   [3] – never

10. Did you have problems getting pregnant in the past?

1 – Yes
2 – No

11. Do you carry any knowledge of a disease that may hinder your chances of getting pregnant (e.g. polysystic ovarian syndrome)

1 – Yes
2 – No

12. If answered yes at question 11 please give a complete description of the disease state

_____

_____

_____

_____

_____

Appendix 4: Questionnaire regarding the subjects attitude to and compliance to the diet

Please answer the following questions with regard to the prescribed diet that where followed over the past 16 weeks

Cross out the option in the block provided

Name:_____

1. **I strictly followed the diet for an average of:**

[1] – one day per week
[2] – two days per week
[3] – three days per week
[4] – four days per week
[5] – five days per week
[6] – six days per week
[7] – seven days per week

2. **The diet was:**

[1] – very acceptable
[2] – acceptable to a certain extend
[3] – unacceptable to a certain extend
[4] – very unacceptable

3. **If you chose option 3 or 4 at question 2 please give a reason**

_____
_____
_____
_____
_____

4. Most of the time I felt:

1 – very hungry
2 – moderately hungry
3 – hungry very few times
4 – not hungry at all

5. I am willing:

1 – to follow the diet in the future, also as a maintenance diet if I reach my target weight
2 – to follow the diet in the future, but only until I have reached my goal weight
3 – to follow the diet some time in the future
4 – never to follow the diet again
5 – Other

6. If you choose option 5 at question 5, please give a reason

_____
_____
_____
_____
_____
_____

Appendix 5: Diet assessment questionnaire

1. Demographic information

1.1 Name_____

1.2 Age:_____

1.3 Chronic medication (Yes/ No)_____

1.4 Systemic contraception (yes/ no)_____

1.5 Brief description of menstrual disorders

_____
_____
_____
_____
_____

2. Anthropometric information:

2.1 Weight (kg)_____

2.2 Height (cm)_____

2.3 Body mass index (kg/m2)_____

3. Level of activity

3.1 Type of exercise _____

3.2 Frequency (times per week)

_____

3.3 Intensity _____

4. Individual eating pattern assessment

4.1 24-hour recall and food frequency questionnaire

Breakfast	
	Eggs **Cheese** **Red meat** **Fish** **Chicken** **Lentils** **Beans**
In between snack	
	Fruit (type) **Fruit juice (type)** **Dried Fruits**
Lunch	
	Vegetables (type) **Bread (type)** **Noodles** **Rice** **Sweet potato** **Corn/ mealies** **Breakfast cereal**
In between snack	
	Margarine (type) **Mayonnaise (type)** **Salad dressing (type)**
Dinner	
	Nuts **Biscuits** **Simba chips** **Pop corn** **Chocolate/ Sweets** **Cold drink (Coke)** **Take away (type)** **Sugar** **Alcohol**
Late night snack	
	Milk (type) **Yoghurt** **Milk powder (type)** **Tea/ Coffee**

Appendix 6: Advertisement in local newspaper Die Volksblad

OVERWEIGHT WOMEN
NEEDED

For participation in a weight reduction research study

The Department of Human Nutrition and Department of Chemical Pathology at the University of the Orange Free State in conjunction with the Reproductive Unit at the Universitas Hospital is conducting a clinical trail:

- **The inclusion criteria for participation in the study is:**

- **Between the ages of 18 to 40 years**
- **Non-smokers**
- **Menstrual abnormalities or difficulty getting pregnant**
- **Willing to follow a prescribed diet for 16 weeks**

Participation in the study will be in conjunction with a registered medical practitioner

For more information contact

Liz-Mare at 083 491 6530

Appendix 7: 250g Carbohydrate test diet (followed 3 days prior to baseline blood tests)

Menu:
Breakfast:
(1 cup of fat free *or* 2% low fat milk) and (1 cup of Cornflakes *or* 1 cup of All Bran flakes *or* 2 slices of bread *or* 2 Weetbix biscuits)
In between snack
(1 slice of bread *or* 3 pro vitas) and 1 teaspoon of margarine and 1 cup of fruit juice
Lunch
(3 slices of bread *or* 1 ½ cup of pasta *or* 1 slice of bread and 1 ½ sachet of Cup of Soup lite + water) and 2 teaspoons of margarine 30g cheese
In between snack
(1 slice of bread *or* 3 provitas) and 1 teaspoon of margarine 1 cup of fruit juice
Dinner
150g of Red meat or chicken or fish and (2 medium potatoes *or* 1 cup of pasta *or* 2/3 cups of rice *or* 1 cup of mealies) 1 teaspoon of margarine ½ cup of boiled carrots *or* peas Mixed greens and tomato salad 2t salad dressing
Late night snack
½ cup of low fat yogurt

1 cup = 250ml and ½ cup = 125ml and 1/3 cup =160ml
1 teaspoon = 5ml

- ½ cup of milk for coffee and tea.
- You can take tomato, cucumber, or Bovril/ Marmite/ Oxo on your bread with snacks
- Please note: No food or drinks (except water) allowed after 22:00 on the day before blood sample collection.

Appendix 8: Telephone screening questionnaire

Section A: Demographic information

1. Age (years)	[18-40years]
2. Weight (kg) – if known	
3. Height (m) -if known	
4. BMI (kg/m^2) - as calculated by interviewer	[>30kg/m^2]
5. Cigarette smoking? (yes/**no**)	
6. Chronic medical condition? (yes/ **no**)	
7. Chronic medicine? (yes/**no**)	

Section B: Menstrual abnormalities

9. Do you menstruate? (yes/ no)	
10. Do you have a regular menstrual cycle? (yes/no)	
11. Do you have a very irregular menstrual cycle (yes/no)	
12. Do you experience very heavy flow during the menstruation phase of your cycle? (yes/ no)	
13. Do you experience spot bleedings between your menstruation phases.	
14. Did you have a difficulty in getting pregnant in the past?	
15. Do you have a diagnosed gynecological problems/ fertility disorder?	

Name:_____

Contact number:_____**(office)**

_____**(after hours)**

DIE UNIVERSITEIT VAN DIE ORANJE-VRYSTAAT
THE UNIVERSITY OF THE ORANGE FREE STATE

Departement Menslike Voeding/Department of Human Nutrition
Fakulteit Gesondheidswetenskappe/Faculty of Health Sciences

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Interne posbus/Internal box 24

CONSENT FORM

Effects of a low-insulin-response, energy restricted diet on weight loss and endocrinological parameters in obese women in their reproductive years.

Ethics committee reference number: 74/00

Declaration on behalf of the participant:

I, the undersigned, ...

[ID.....]

..... (address)

.....(telephone number)

A confirm that:

1. I have been asked to take part in the above-mentioned research study carried out by the Department of Human Nutrition at the University of the Orange Free State.

2. It has been explained to me that:

2.1 The purpose of the research is to determine the effect of a low-insulin-response diet on certain blood parameters in obese women. The results of the study will show if the low-insulin-response diet can effectively be used in the fertility treatment of obese women.

2.2 In order to carry out the study I have been told that I will be asked a number of questions regarding general background information.

2.3 I also understand that blood samples will be drawn by a medical doctor. I also agree to be weighed, measured and have my body fat percentage taken. I will not eat or drink anything after 22:00 of the evening preceding the day that the blood will be drawn. I will provide a list of the medication that I usually use on the research day.

2.4 I have been told that these information will be collected from 40 women in Bloemfontein and that measurements and blood samples will be taken on the first and last day of the research study. I understand that I will follow the diet for 12-20 weeks. I have further been told that I am expected to come for a weekly weighing session and that I will further be expected to keep a daily temperature card and food record.

3. I have been told that due to the nature of the study I will be advised not to use systemic contraceptives (e.g. pill and injection) and if I choose to start using systemic contraception I will inform the researchers. It has further been stated that condoms will be available at no cost on each weekly weighing session.

4. I have been told that I will be considered a dropout in the study if I miss more than two consecutive weighing sessions, if I start to use systemic contraception, fail to complete the daily food record and temperature card or jeopardise the study in any way. I will be withdrawn from the study if I become pregnant.

5. I have been told that the study will be conducted over 12-20 weeks (3-5 months).

6. I have been told that the measurements will not cause harm to me in any way.

7. It was also explained to me that all information will be kept confidential but that it will be used anonymously for making known the findings to other scientists.

8. I understand that I will have access to the results through contact with the researcher who will inform me with the findings.

9. I was also explained to me that participation in the study is voluntary and that I may withdraw from the study at any time.

10. The information in this consent form was explained to me by.....(name of the interviewer) in ..... (language) and that I confirm that I have a good command in this language and understand the explanations. I was also given the opportunity to ask questions on things I did not understand clearly

11. No pressure was applied on me to take part in the research study.

B I hereby agree voluntarily to take part in this research study.

Signed/ confirmed at ..... on 2000

.....
Signature of participant

.....
Signature of witness

Appendix 10: DATA RECORD FORM

Name: _____

Subject's number: _____

Group: _____

Name of data collector: _____

Bodystat® no: _____

Anthropometrical data:

Parameter	Baseline	End of trail
Weight (kg)		
Height (m)		
BMI (kg/m^2)		
Body fat percentage		
Body fat mass (kg)		
Lean muscle percentage		
Lean muscle mass (kg)		
Body water %		
Body water mass (L)		
Middle circumference (cm)		
Hip circumference (cm)		
WHR		

Comments: _____

Appendix 11: Daily food intake record form

Subject's name: Subject's number: week: Date:

	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Breakfast:							
In between:							
Lunch:							
In between:							
Dinner:							
Late night:							

SUMMARY

There is consistent evidence that obese women are less fertile than women of normal body weight. Obesity, in particular android obesity, is associated with several sex steroid abnormalities in premenopausal women including: increased free estrogen and androgen fractions, reduced sex hormone-binding globulin and increased bio-active estrogen delivery to target tissue.

The state of insulin resistance with secondary hyperinsulinemia is commonly observed in obese, infertile women whereas the gonadotrophic effects of insulin on ovarian steroid hormone synthesis have been indicated *in vivo* and *in vitro*. Insulin can directly and indirectly stimulate ovarian androgen production. The exaggerated insulin action on ovarian tissues may present the pathological mechanism for disturbances in the endocrine profile and menstrual cycle and infertility in some obese women.

Weight loss is associated with a significant improvement in menstrual abnormalities, ovulation and fertility rates with a reduction in hyperandrogenism and hyperinsulinemia. It is suggested that weight loss should be the first option in the treatment of overweight infertile women. Intervention studies suggest that reducing weight and/or hyperinsulinemia either by diet alone or a combination of diet and drug therapy should be investigated.

This study was undertaken to evaluate the effects of a low-insulin-response, energy-restricted diet (LID) on anthropometric and endocrinological parameters in obese women with menstrual abnormalities. For the purpose of this study we compared the effects LID to a normal balanced-energy restricted diet. The principles for the LID were based on the available literature regarding the insulin response to foods and their combinations.

At baseline 37 candidates were randomly assigned into two groups. Group A followed the LID and consisted of 19 candidates whereas Group B followed the ND and consisted of 18 subjects. The inclusion criteria were: obese (BMI > $30kg/m^2$), premonopausal, insulin resistance, anovulation and between the ages of 18 and 04 years of age whereas the exclusion criteria included increased fasting and stimulated glucose concentrations, cigarette-smokers, and the presence of any chronic medical condition. Subject fasted for a 10 to 12 hour period after following a 250 g carbohydrate diet for three-days prior to baseline blood sampling. Blood samples were collected at baseline and at the end of the 16-week trial and analyzed for fasting insulin and glucose, testosterone (T), luteinizing hormone (LH), follicle stimulating hormone (FSH), estrogen (E), prolactin, thyroid stimulating hormone (TSH), thyroxine (FT4), leptin and progesterone. Insulin resistance were defined as a glucose-to-insulin ratio < 4.5. Stimulated 30 and 120-minute insulin and glucose were collected after subjects consumed 82.5g of monohydrate glucose powder diluted in 300ml water.

Due to certain limitations we did not diagnose anovulation whereas only a certain percentage of the subjects in each group were hyperinsulinemic. One of the problems experienced was the drop outs in each group.

Results form this trial indicated a significant reduction in fasting and 30-minute stimulated insulin, LH, and testosterone and leptin concentrations in the ND group whereas only leptin concentrations reduced significantly in the LID group. A significant reduction occurred in mean weight, BMI, body fat percentage, waist and hip circumference in both groups.

Numerous studies evaluated the effects of weight loss on fertility but to our knowledge this is the first trial of its kind to evaluate the effects of two different test diets on fertility parameters. Results from this trial confirm the positive effects of weight loss on endocrinological and anthropometric parameters in obese women, however, the LID showed no beneficial effects over the ND. Future research is needed to evaluate the effect of diet manipulation on fertility parameters with specific regard to diet manipulation in combination with drug therapy. This trial, however, serves as a good pilot study for future research of this kind.