From the Department of Woman and Child Health
Division of Obstetrics and Gynecology
Karolinska Institutet, Stockholm, Sweden

ENDOCRINE AND METABOLIC DISORDERS IN BULIMIC WOMEN AND EFFECTS OF ANTIANDROGENIC TREATMENT

Sabine Naessén

Karolinska Institutet

Stockholm 2006

Cover: “Weeping Woman; The Hungry Ghost” Pablo Picasso 1937

All previously published papers were reproduced with permission from the publisher.
Published by Karolinska Institutet. Printed by Repro Print AB, Stockholm 2006

ISBN 91-7357-003-6

To my beloved daughter Ida

ABSTRACT

Background: Bulimia is a mental disorder frequently associated with menstrual disturbances and low estradiol levels although most bulimic women are of normal weight. Low bone mass has also been reported in these women. Furthermore, increased androgen levels and polycystic ovaries (PCO) have been described in bulimic women. Little is known about the mechanisms of these hormonal disturbances and the role of sex hormones in the etiology of the disease has not been fully explored. Androgens may promote bulimic behavior by influencing food craving or impulse control.

Aims: The specific aims of this work were to compare women with bulimia to healthy controls with respect to: 1) menstrual disturbances and the occurrence of polycystic ovary syndrome (PCOS) 2) endocrine and nutrition-related factors predicting bone mass 3) estrogen receptor (ER) ß polymorphism in view of the potential role of estrogen signaling in bulimic disease 4) effects of an antiandrogenic oral contraceptive (OC) on appetite and eating behavior.

Methods: Seventy-seven bulimics and 59 controls were investigated with respect to menstrual status, PCO, symptoms of hyperandrogenism, bone mineral density (BMD), sex hormone levels and ERß polymorphism. Meal-related appetite response and bulimic symptoms were evaluated in 21 women with bulimia nervosa before and after three months of treatment with an antiandrogenic OC.

Results: 1) Bulimics had a higher occurrence of menstrual disturbances, hirsutism and PCOS than controls. Positive correlations between hirsutism and levels of biologically active testosterone were found in bulimics but not in controls. 2) Bulimics had a lower spinal BMD and higher frequency of osteopenia in the total body than controls. Subgroups of bulimics with a history of amenorrhea or previous anorexia nervosa had the lowest BMD values, whereas those without such history did not differ from controls. Multiple regression analysis including significant endocrine and nutrition-related variables, revealed previous anorexia nervosa to be the strongest determinant of spinal BMD in bulimic women. 3) An association was found between two common polymorphisms in the ERβ gene and bulimic disease. A novel variant changing the primary structure of ERβ protein was identified in one bulimic patient, but an initial characterization of this variant did not reveal any differences compared to the wild type protein. 4) Antiandrogenic OC treatment reduced meal-related hunger and gastric distention in women with bulimia nervosa and improved bulimic behavior in relation to reduced testosterone levels.

Conclusions: 1) Our study supports an increased frequency of PCOS in bulimic women. This endocrine disorder may be of importance for the development of bulimia. 2) Low bone mass in bulimics could be explained by previous anorexia nervosa, whereas bulimia *per se* does not influence bone mass negatively. 3) Genetic variation in ERβ may play a role in the etiology of bulimic disease. 4) An antiandrogenic OC may develop into a new strategy for treatment of women with bulimia nervosa not responding to conventional therapy and particularly in those with hyperandrogenic symptoms.

LIST OF PUBLICATIONS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals I-IV:

I. Naessén S, Carlström K, Garoff L, Glant R, Hirschberg AL. Polycystic ovary syndrome in bulimic women – an evaluation based o the new diagnostic criteria. Gynecol Endocrinol 2006;22:388-394.

II. Naessén S, Carlström K, Glant R, Hans Jacobsson and Hirschberg AL. Bone mineral density in bulimic women – influence of endocrine factors and previous anorexia. European J Endocrinol 2006;155:245-251.

III. Nilsson M, Naessén S, I Dahlman, Hirschberg AL, Gustafsson J-Å and Dahlman-Wright K. Association of estrogen receptor β gene polymorphisms with bulimic disease in women. Molecular Psychiatry 2004 ;9:28-34.

IV. Naessén S, Carlström K, Byström B, Pierre Y and Hirschberg AL. Effects of an antiandrogenic oral contraceptive on appetite and eating behavior in bulimic women. Under revision.

CONTENTS

ABBREVIATIONS

A-4	4-androstene-3, 17-dione
ACTH	Adrenocorticotropic hormone
AN	Anorexia nervosa
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
APA	American psychiatric association
ASRM	American Society of Reproductive Medicine
BITE	Bulimia investigation test
BMD	Bone mineral density
BMI	Body mass index
BN	Bulimia nervosa
CA	Dinucleotide cysteine/adenine repeat
CBT	Cognitive-behavioral therapy
CCK	Cholecystokinin
CEIA	Chemoluminiscence enzyme immunoassay
CMR	Crude mortality rate
CV	Coefficient of variation
DXA	Dual energy X-ray absorptiometry
DHEAS	Dehdroepiandrosterone sulfate
DIAB	Diagnostic interview for anorexia and bulimia
DNA	Deoxyribonucleic acid
DRSP	Drospirenone
DSM-IV	Diagnostic and statistical manual of mental disorders, ed 4
E2	Estradiol-17-β
EDNOS	Eating disorders not otherwise specified
ER	Estrogen receptor
ESHRE	European Society for Human Reproduction
FIRI	Fasting insulin resistance index
FSH	Follicle-stimulating hormone
fT4	Free thyroxine
HPA	Hypothalamic-pituitary-adrenal axis
HPG	Hypothalamic-pituitary-gonadal axis
HPLC	High performance liquid chromatography
IGF-I	Insulin like growth factor-I
IRP	International reference preparation
ISDC	International Society for Clinical Densitometry
LH	Luteinizing hormone
OC	Oral contraceptive
OR	Odds ratio
PCO	Polycystic ovaries
PCOS	Polycystic ovary syndrome
PCR	Polymerase chain reaction
PMS	Premenstrual symptoms
PSR	Psychiatric status rating scale
RANKL	Receptor activator of NF-κB ligand
RIA	Radioimmunoassay

SD	Standard deviation
SHBG	Sex hormone-binding globulin
SNP	Single nucleotide polymorphism
SSRI	Selective serotonin reuptake inhibitor
TMHA	Temperature modulated high performance liquid chromatography
TSH	Thyroid-stimulating hormone
VAS	Visual-analogue scale
WHO	World Health Organization

1 INTRODUCTION

1.1 BULIMIA – AN EATING DISORDER

1.1.1 Background and clinical features

The word bulimia comes from the Greek *boulimia* meaning "the hunger of an ox" and depicts the extreme nature of binge eating. The symptoms of bulimia are described by the ancient Egyptians and in the Talmud. The Egyptians believed that diseases came from food and purged on a monthly basis. Binge eating was widely practiced during the Antiquity and wealthy Romans had “vomitoriums”, pleasant rooms for purging between courses. During the Middle Ages, religious authorities considered gluttony a mortal sin. Self-induced vomiting was allowed as penance. Forced emesis was also practiced among monks to control sexual drives and sharpen intellect. Bulimia was first described in Sweden by Carl von Linné (1707-1778). In the later half of the twentieth century eating disorders were identified as widespread cultural phenomena.

Bulimia nervosa (BN) was first defined by Professor Gerald Russell in 1979 and in 1980 BN was recognized as an autonomous eating disorder by the American Psychiatric Association (*APA 1980*). BN is a mental disorder characterized by self-perpetuating and self-defeating cycles of binge-eating and the regular use of inappropriate compensatory behavior in order to prevent weight gain. During a "binge," the person consumes a large amount of food in a rapid, automatic and uncontrolled fashion (*Fairburn & Cooper 1984; Walsh et al. 1992*). This may anesthetize hunger, tension, anger and other feelings, but it eventually creates physical discomfort and anxiety about weight gain. Thus, the person "purges" the food eaten, usually by inducing vomiting or by the misuse of laxatives and diuretics. The individual may also resort other compensatory behaviors, such as restrictive dieting and excessive exercise (*Kreipe et al. 2000*).

The prevalence rate of BN is 1-2% in young females (*Fairburn & Beglin 1990; Rodriguez-Cano et al. 2005*) and bulimia is 10 - 20 times more common in women than in men (*Hoek et al. 1995; Woodside et al. 2001*). Unfortunately, because of the denial, embarrassment, shame, and secrecy associated with bulimia, the illness can often go unacknowledged, delaying assessment and intervention. The reported prevalence rate of BN can therefore only serve as a minimum estimate of the true prevalence.

BN most commonly occurs in women who are of normal body weight and onset is usually during the period from mid adolescence through the mid-twenties. Bulimic women have several symptoms in common with patients having anorexia nervosa (AN), such as a drive to restrain their food intake, an extreme fear of weight gain and often a distorted image of their bodies. However, abnormally low body weight excludes the diagnosis of BN. Twenty-five to 30% of individuals with BN have a prior history of AN, whereas some 5% of women with BN will eventually develop AN (*Kaye et al. 2000*). The similarities between these two disorders have suggested at least some risk and liability factors in common.

Many patients with bulimia have a coexisting psychiatric condition, such as an anxiety disorder or depression (*O'Brien & Vincent 2003*). Major depression is commonly noted, although it is not clear if the mood disturbance is a function of BN or a separate phenomenon (*Mury et al. 1995; Blinder et al. 2006*). There is also an association with abuse of alcohol and stimulants and promiscuity (*Neumark-Sztainer et al. 1996*). Furthermore, different personality disorders, such as borderline, histrionic, narcissistic and antisocial personality disorder are commonly coexisting with BN (*Sansone et al. 2005; Lilenfeld 2006*).

BN has a chronic, sometimes episodic course in which periods of remission alternate with recurrence of binge/purge cycles. Loss of control of eating typically occurs only some time after the onset of dieting behavior. There may be spontaneous recovery without treatment, recovery after any of a variety of treatments and a fluctuating course of weight gains followed by relapses (*Fairburn et al. 2000*). The long-term course and outcome of BN has not been determined. Available data indicate that about 35% of patients with BN rapidly relapse and up to 40% remain chronically symptomatic (*Keel*

et al. 2005; Olmsted et al. 2005). Psychiatric co-morbidity such as having a personality disorder seems to be the best predictor for eating disorder outcome (*Vaz 1998; Fichter & Quadflieg 2004*). The risk of death is much lower among patients with BN than among those with AN (*Steinhausen & Verhulst 1999*) but appears to be greater than that among women of similar age in the general population (*Keel & Mitchell 1997*). The crude mortality rate (CMR) based on meta-analyses of BN, reveals a CMR of 0.4% (*Nielsen 2001*).

1.1.2 Etiology

The etiology of bulimia is unknown but genetic, biological, social and psychological factors all seem to play a role (*APA 1994; Bulik et al. 2003*).

Familial factors may increase the risk of developing the disorder. First- and second-degree relatives of individuals with BN have an increased incidence of depression and manic-depressive illnesses, eating disorders, and abuse problems (*Lilenfeld et al. 2000; Duncan et al. 2005*). The involvement of a genetic component in the etiology of bulimic disease is suggested from studies of families with bulimic patients and twins (*Holderness et al. 1994; Lilenfeld et al. 1998; Silberg & Bulik 2005*). However, the actual genes involved in the development of disease remain to be elucidated.

Abnormalities of central nervous system neurotransmitters which regulate mood and satiety, especially serotonin, may also play a role in BN (*Kaye et al. 1992*). Women with BN are known to have alterations of brain serotonin activity and mood, as well as obsessions with perfectionism. These alterations and symptoms seem to persist after recovery from BN, suggesting that they are not merely a consequence of abnormal eating behavior (*Kaye et al. 2001*). Theoretically, altered serotonin activity could cause anxiety and obsession and affect the control of appetite and thus contribute to a vulnerability to develop BN (*Kaye et al. 2001*).

Appetite regulating peptides may also be involved in the development and maintenance of bulimia. Cholecystokinin (CCK), which is present in both gut and brain, is released in response to food intake and exerts well-known gastrointestinal effects such as stimulation of gall bladder contraction, pancreatic exocrine secretion and inhibition of gastric emptying (*Grider 1994; Di Franco et al. 2005*). Moreover,

exogenously administered CCK reduces food intake both in experimental animals and in man (*Gibbs & Smith 1994*). Animal experiments suggest that CCK has both a peripheral action, since it activates abdominal vagal afferents and a central action on feeding centers in the brain (*Gibbs & Smith 1994*). In humans, peripheral blood levels of CCK have been shown to have a positive association with subjective satiety (*Hirschberg et al. 1994*), and there is now substantial evidence for CCK being a physiological satiety peptide. It has been demonstrated that bulimic patients have an impaired meal-related secretion of CCK related to decreased satiety (*Devlin et al. 1997*). Ghrelin, on the other hand, is an appetite-stimulating peptide leading to increased food intake both in animals and humans (*Wren et al. 2001*). The primary source of ghrelin is the stomach and the duodenum, but it is also produced in the pituitary, hypothalamus, liver and kidneys (*Buczkowska 2005*). Circulating ghrelin rises before a meal and falls after a meal, suggesting a possible role in the induction of a meal (*Cummings et al. 2001*). In bulimics, ghrelin levels have been reported to be increased (*Tanaka et al. 2002; Monteleone et al. 2003*), as well as comparable to controls (*Nakazato et al. 2004; Monteleone et al. 2005*).

Furthermore, the development of an eating disorder is often attributed to our cultural environment, as exposed in mass media, which place a heavy emphasis on slimness. However, only a small percentage of all women who are exposed to these cultural mores develop eating disorders. Other predisposing factors include perfectionism, impaired self-esteem, affective instability, poor impulse control and absence of adaptive functioning to maturational tasks and developmental stressors, e.g. puberty, peer and parental relationships, sexuality, marriage and pregnancy.

There is no evidence of a relationship between bulimia and socio-economic status (*Rodriguez et al. 2004*). However, some occupations appear to run a greater potential risk of being linked to the development of an eating disorder. Typical examples of these are professions within the world of fashion and dance (*Vandereycken & Hoek 1993; Santonastaso et al. 2002*). Eating disorders are also common among female athletes (*Sundgot-Borgen & Torstveit 2004*). However, BN and binge eating disorders are most likely to develop in dieters who are at risk of obesity and psychiatric disorders in general (*Fairburn et al. 1998; Hudson et al. 2006*).

1.2 TREATMENT OF BULIMIA

At present, the treatment of choice for BN is cognitive-behavioral therapy (CBT), which focuses on restructuring the maladaptive thoughts and behaviors that provoke binge eating and purging (*Hay & Bacaltcuk 2001*). CBT can be administered either individually or in groups. The vast majority of patients can be managed safely as out-patients; however risk for suicide and other high-risk behaviors and medical comorbidity should be identified at assessment. In addition to CBT, antidepressant therapy can be necessary in many cases. Antidepressant medications in the selective serotonin reuptake inhibitor (SSRI) class, such as fluoxetine, have been shown to reduce the frequency of binge eating and purging and are effective also in patients who are not depressed (*Leombruni et al. 2006*). However, there are limited data on long-term effectiveness and a subset of patients with bulimia does not respond adequately to CBT, antidepressant medication or both. New treatment approaches for bulimia are therefore needed.

1.3 MEDICAL COMPLICATIONS

Medical complications have an important clinical role in patients with eating disorders. The vomiting and the laxative or diuretic abuse in bulimics can result in hypochloremia, hypokalemia and metabolic alkalosis, which can be most severe and in rare cases, result in sudden death due to arrhythmias (*Mitchell & Crow 2006*). Determination of electrolyte levels is therefore crucial in the evaluation of the disorder.

Most patients have gastrointestinal signs and symptoms. Vomiting frequently results in esophageal irritation and chest pain. Other symptoms related to binge-eating and vomiting are salivary gland hypertrophy, erosion of tooth enamel, abdominal pain, bloating and constipation. Increased gastric capacity and delayed gastric emptying (*Hadley & Walsh* 2003) have been reported in bulimic patients. Some patients may develop calluses on their knuckles, known as Russell sign, from biting on their fingers when they vomit. Biochemical abnormalities related to gastrointestinal disturbances are increased liver enzymes and elevated cholesterol levels.

Endocrine disorders in bulimic women include amenorrhea/oligomenorrhea. Menstrual disturbances can play a role in the development of osteopenia and ultimately osteoporosis in some patients who have bulimia. These disorders are described in more

detail below. Figure 1 illustrates the variety of medical complications in women with bulimia.

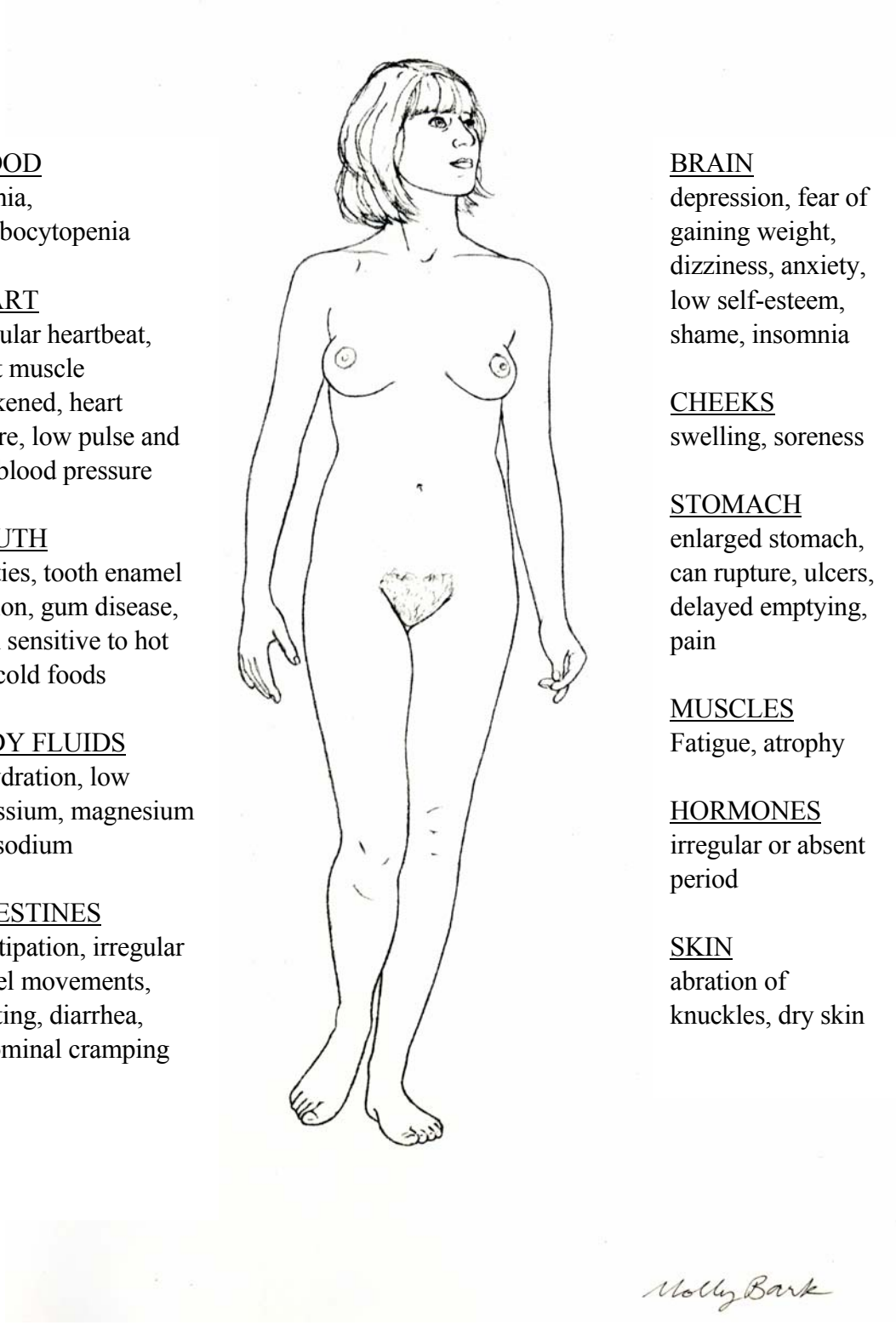

Figure 1. Medical symptoms in bulimic women.

1.3.1 Menstrual disturbances in bulimic women

Menstrual dysfunction was identified as a complication of BN in the earliest description of the illness (*Russel 1979*). Despite maintenance of normal body weight, the occurrence of amenorrhea in bulimic women has been reported within the range of 7-40% (*Fairburn & Cooper 1982; Copeland & Herzog 1987; Copeland et al. 1995*) and 37-64 % of the patients may have oligomenorrhea (*Cantopher et al. 1988*). In comparison, the prevalence of secondary amenorrhea in the general population is between 2-5% (*Münster et al. 1992*).

Even though menstrual disturbances are common among bulimic women (*Abraham 1998; Crow et al. 2002; Gendall et al. 2000*), the mechanisms are not fully understood. In AN, the usual cause of menstrual disturbance is hypothalamic amenorrhea due to starvation (*Golden & Shenker 1994; Chan & Mantzoros 2005*). Low levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), indicating hypothalamic inhibition of the pituitary-gonadal axis have also been reported in bulimic women (*Schweiger et al. 1992; Cotrufo et al. 2000*). Furthermore, low levels of thyroid hormones, considered to be a consequence of the temporary starvation periods associated with the disease, have been reported in BN (*Schweiger et al. 1992; Altemus et al. 1996; Gendall et al. 2000*).

Different mechanisms may interact in the etiology of menstrual disturbances in bulimia. An increased occurrence of polycystic ovaries (PCO) (*McCluskey et al. 1992; Jahanfar et al. 1995; Raphael et al. 1995*), acne (*Gupta et al. 1992; McSheery 1992*) and elevated serum levels of testosterone (*Sundblad et al. 1994; Cotrufo et al. 2000; Monteleone et al. 2001*) have been reported in bulimic women. These observations led to the suggestion of a link between bulimia and polycystic ovary syndrome (PCOS) (*McCluskey et al 1991; McCluskey et al. 1992; Jahanfar et al. 1995; Raphael et al. 1995; Morgan et al. 2002; Hirschberg et al. 2004; Moran & Norman 2004*).

PCOS is the most common hormonal aberration in women of fertile age, with a prevalence of 5-10%, and is associated with chronic anovulation, clinical symptoms of hyperandrogenism and PCO morphology (*Ehrmann 2005*). Furthermore, the syndrome is often associated with insulin resistance and abdominal obesity (*Essah & Nestler 2006*). Like in bulimia, PCOS patients have disturbed appetite regulation (*Hirschberg*

et al. 2004; Moran et al. 2004) and an increased score of the bulimia investigation test (BITE) has been reported in these patients (*McCluskey et al. 1991*). The etiology of PCOS is not fully elucidated and several factors seem to interact in the development of the disorder. Family studies of patients with PCOS suggest a strong genetic basis for the syndrome (*Carey et al. 1993; Franks et al. 1997; Moran & Norman 2004*).

The definition of PCOS has generated much debate and confusion through the years. Recently, the diagnostic criteria for the diagnosis of PCOS were revised on the 2003 Rotterdam consensus workshop sponsored by the European Society for Human Reproduction (ESHRE) and the American Society of Reproductive Medicine (ASRM) (*Rotterdam consensus 2004*). The criteria are:1. oligo- or anovulation, 2. clinical and/or biochemical hyperandrogenism and 3. PCO on ultrasound. Two out of these three criteria are necessary for diagnosis.

There is still a great uncertainty about the causes behind the increased frequency of menstrual disturbances in bulimic women and the proposed association between bulimic behavior and PCOS has been questioned (*Chapdelaine 1991; Michelmore et al. 2001*). It is therefore important to explore the endocrine mechanisms for menstrual disturbances in bulimic women.

1.3.2 Bone mass in women with bulimia

Low bone mineral density (BMD) in women with eating disorders is well established. Over the years, several studies have investigated BMD in bulimic patients. However, these studies have produced conflicting results showing normal (*Newman & Halmi 1989, Sundgot-Borgen et al. 1998; Zipfer et al. 2001*), as well as low BMD in bulimic women (*Howat et al. 1989; Joyce et al. 1990; Newton et al. 1993; Baker et al. 2000*), in the latter case indicating a risk for osteoporosis in this patient group.

It is well known that 25-30% of patients with bulimia have a previous history of AN (*Fairburn & Hope 1988; Kaye et al. 2000*). AN is associated with an apparent loss of bone mass, particularly of trabecular bone and up to 50% of anorectic patients have osteoporosis in the lumbar spine (*Legroux-Gerot et al. 2005*). Bone loss in AN is considered to be secondary to a catabolic state caused by restrictive eating, a low calcium intake and estrogen deficiency leading to amenorrhea (*Katzman 2003;*

Legroux-Gerot et al. 2005). Recovery from the illness (weight gain, regular menstruations) does not always compensate for an early bone loss (*Kaplan et al. 1986; Bachrach et al. 1991; Legroux et al. 2005*).

Eating disorders often start during adolescence, which is a critical time in bone metabolism as most bone mineral is accumulated during the teenage years (*Bonjour et al. 1991*). Eating disorders during this time period may therefore preclude the woman to reach her full peak bone mass. Osteoporosis in women is often a reflection of low peak skeletal mass attained and maintained in young adulthood (*Recker et al. 1992; Anderson et al. 1996*). Clinical risk factors for osteoporosis include low minimum body mass index (BMI) during this period of life and long duration of AN and amenorrhea (*Rigotti et al. 1991; Rivera-Gallardo et al. 2005*).

Several endocrine abnormalities in bulimic women may have adverse effects on bone mass. Low levels of estradiol leading to menstrual disturbances are common (*Abraham 1989; Gendall et al. 2000; Crow et al. 2002; Monteleone et al. 2002*). Estadiol is crucial for maintenance of bone mass (*Migliaccio et al. 1996; Lerner 2006*). The mechanisms involve specific estrogen receptor actions regulating bone remodeling, resorption and formation. Estrogen inhibits bone resorption via effects on the receptor activator of NF-κB ligand (RANKL) and osteoprotegerin production, as well as by reducing the production of number of pro-resorptive cytokines, along with direct effects on osteoclast activity and lifespan (*Syed & Khosla 2005*). The effects of estrogen on bone formation include a prolongation of osteoblast lifespan via non-genotropic mechanisms, as well as effects on osteoblast differentiation and function (*Syed & Khosla 2005*). Suppression of osteoclastic bone resorption and stimulation of osteoblastic bone formation thus form the basis for the bone-preserving effects of estradiol.

Elevated circulating cortisol and ACTH concentrations (*Levy 1989, Mortola et al 1989; Bailer & Kaye 2003*) have also been reported in BN, suggesting an increased activity of the hypothalamic-pituitary-adrenal (HPA) axis. This may be related to a catabolic state created by the temporary starvation periods related to this disease. However, another reason may be the partial glucocorticoid insensitivity of the HPA-axis in bulimics as indicated by many reports about a poor response to dexamethasone

in this disease (*Levy 1989; Neudeck et al. 2001*). Hypothetically, this may lead to a compensatory increase in ACTH secretion and thus to increased cortisol levels. Hypercortisolism is well-known to affect the skeleton negatively and may eventually lead to osteoporosis (*McIlwain 2003*). Furthermore, low levels of thyroid hormones, also considered to be a consequence of the temporary starvation periods, have been demonstrated in BN (*Schweiger et al. 1992; Altemus et al. 1996; Gendall et al. 2000*). Thyroid hormones are essential to maintain skeletal growth and remodeling (*Laron 2003; Galliford et al. 2005*). Insulin and insulin-like growth factor-I (IGF-I) are two factors closely related to nutritional status. IGF-I has anabolic effects on bone mass (*Rosen & Bilezikian 2001*). However, normal insulin status (*Russell et al. 1996*) and normal IGF-I levels (*Levy & Malarkey 1988*) have been reported in BN.

It is still unclear whether bulimia is associated with low bone mass or not and the influence of endocrine factors and of previous AN on BMD in this patient group has not been fully explored.

1.4 SEX HORMONES AND EATING BEHAVIOR

Eating disorders are much more common in women than in men (*Andersen 1999*), suggesting a possible role for sex hormone signaling in the etiology of bulimia. In females of many species, feeding is closely associated with hypothalamic-pituitary-gonadal (HPG) axis function. Rodents and primates eat less during estrus (time prior to and following ovulation when animals have increased sexual receptivity). A similar decrease in eating during the ovulatory phase in cycling women has been demonstrated (*Marchal-Victorion et al. 2002; Olszewski et al. 2003*). Estrogen is known to inhibit feeding in animal experiments (*Lindén et al. 1990; Geary 2001*) and to increase the activity of the CCK satiation signaling pathway (*Andersen 1999*). The satiating potency of endogenous CCK is increased during estrus in normal rats and by estradiol treatment in ovariectomized rats (*Lindén et al. 1990; Asarian & Geary 1999; Eckel & Geary 1999*). Little is, however, known about the role of low levels of estradiol for bulimic behavior in women.

In the hypothalamus, a brain area known to be involved in the regulation of appetite and satiety, both estrogen receptor (ER) α and ERβ are expressed (*Laflamme et al. 1998*). Recently, ERβ was found to be the receptor regulating the anorectic action of

estrogen in mice (*Liang et al. 2002*). Until now only a few variants of the ERß gene have been reported and characterized with regard to allele frequency (*Rosenkranz et al. 1998; Ogawa et al. 2000a; Ogawa et al. 2000b; Sundarrajan et al. 2001*). Polymorphisms in the ERβ gene have been associated with ovulatory dysfunction (*Sundarrajan et al. 2001*), hypertension (*Ogawa et al. 2000a*) and with high BMD (*Ogawa et al. 2000b*). However, the role of variations in the ERβ gene in bulimic disease is not known.

Testosterone is known to stimulate appetite (*Earley & Leonard 1979*) and to impair impulse control (*Eriksson et al. 1992*). These effects of testosterone are considered to be centrally mediated (*Higley 1996*). Women with high androgen levels and PCOS have a greater craving for sweets and a tendency of binge-eating (*Hirschberg et al. 2004; Klein 2006*). Furthermore, recovery from bulimia has been associated with normalization of ovarian morphology on ultrasound (*Morgan et al. 2002*). It has therefore been suggested that high androgen levels may promote bulimic behavior by influencing food craving or impulse control. However, the proposed association between bulimic behavior and PCOS has been questioned (*Chapdelaine 1991; Michelmore et al. 2001*). In a cross-sectional observational study of 230 women in the general population, Michelmore and co-workers found no significant association between PCO on ultrasound with binge-eating or overeating (*Michelmore et al. 2001*). Further studies are needed to clarify the impact of androgens in bulimic behavior in women.

1.5 INFLUENCE OF SEX HORMONE TREATMENT ON APPETITE AND EATING BEHAVIOR

Cognitive-behavioral therapy in combination with antidepressive medication is currently the standard treatment for bulimic women, but several cases are resistant to this therapy. Furthermore, the risk of relapse is high (about 35%) (*Keel et al. 2005; Olmsted et al. 2005*). Thus, there is a need for additional treatment approaches for bulimia and relapse prevention strategies.

The associations between endocrine status and eating behavior presented above may suggest that treatment with estrogenic and/or antiandrogenic activity may be effective as an additional therapy for bulimic women. In support of this hypothesis, treatment

with the androgen receptor antagonist flutamide has been shown to reduce symptoms in bulimic patients (*Bergman & Eriksson 1996; Sundblad et al. 2005*). However, this medication has been associated with adverse liver effects (*Osculati & Castiglioni 2006)*, which would limit the long-term use of the drug.

Oral contraceptives (OCs) containing the combination of an estrogenic and a gestagenic compound are used by numerous women for birth control, but OCs are also frequently used for medical treatment. Dysmenorrhea, bleeding disorders, endometriosis, hypogonadism and PCOS are common indications for treatment with OCs. The treatment is generally well tolerated with few side-effects. However, there is a low risk for thromboembolic events. Some women may experience changes in appetite and weight, although studies evaluating body weight, all show no significant change in average weight and body composition (*Elger et al. 2003; Milsom et al. 2006*). Despite extensive clinical experience of OC treatment, effects on appetite and metabolism still remain to be explored.

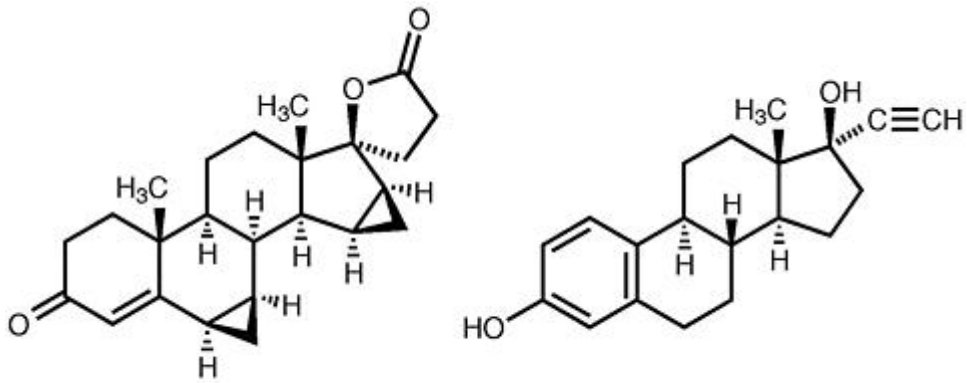

Figure 2. Chemical structure of drospirenone and ethinyl estradiol in Yasmin®.

Yasmin® is a relatively new monophasic OC containing 30 µg ethinyl estradiol and 3 mg drospirenone (DRSP). DRSP (6β, 7β, 15β, 16β-dimethylene-3-oxo-17 α-pregn-4-ene-21, 17carbolactone) is the first synthetic progestin with antimineralocorticoid activity together with considerable antiandrogenic effects (*Foidart 2000; Sitruk-Ware 2005*) and thus differs from other synthetic progestins currently used in OCs. The antimineralocorticoid effect of DRSP may help prevent sodium retention and a rise in blood pressure in susceptible women (*Oelkers 2004; White et al. 2005; Sica 2006*).

Furthermore, a small reduction in body weight has been demonstrated in users of Yasmin® (*Elger et al. 2003*). DRSP exerts direct antiandrogenic activity due to competitive binding to the androgen receptor (*Fuhrmann et al. 1996; Sitruk-Ware 2005*). In addition, DRSP alone or in combination with ethinyl estradiol will suppress androgen production from the adrenals and ovaries (*Muhn et al. 1995; Fuhrmann et al. 1996; Krattemmacher 2000*). Yasmin® has been successfully used for treatment of hyperandrogenic symptoms in women with PCOS (*Pekhlivanov et al. 2006*).

Theoretically, the antiandrogenic and estrogenic properties of Yasmin® may be beneficial for bulimic patients by influencing food craving or impulse control. The effects of this OC on appetite and eating behavior in women with BN have not been studied.

2 AIMS

Bulimia is associated with menstrual disturbances although most bulimic women are of normal weight. Low bone mass has also been reported in these women. Little is known about the mechanisms of such medical complications. Furthermore, the role of sex hormones in the etiology of the disease has not been fully explored. The general aim of the thesis was to study endocrine and metabolic disorders in bulimic women and effects of antiandrogenic treatment.

The specific aims of this thesis were:

- To explore endocrine mechanisms for menstrual disturbances in bulimic women and the potential association between bulimia and PCOS.
- To elucidate possible endocrine and nutrition-related factors predicting bone mass in women with bulimia.
- To study ER-ß gene variants in bulimic women compared to controls.
- To evaluate the effects of an antiandrogenic oral contraceptive on meal-related appetite response and eating behavior in women with bulimia nervosa.

3 MATERIAL AND METHODS

3.1 SUBJECTS

Patients and controls were recruited by media advertisements and via written information about the study which was distributed among students and hospital staff. The general inclusion criteria for the subjects studied in papers I – III were age 18-40 years, BMI 19-33, of general health with exception of eating and menstrual disorders and no medications. They should not have taken hormones, OCs or psychotropic drugs at least three months before the study. Controls should be matched for age and BMI and not have any history of eating disorder.

Approximately 250 women were screened by telephone interview. Several subjects did not fulfill the inclusion criteria or were not willing or able to participate. Usage of OCs was a reason for exclusion in many cases. The women were not recruited according to menstrual status. One-hundred and fifty-two potential bulimics and controls were interviewed by a trained research psychologist using a semi-structured clinical interview DIAB 3.0, Diagnostic interview for anorexia and bulimia (*Clinton & Glant 1992*) generating data for a diagnosis based on the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) (*APA 1994*). Diagnostic criteria for BN, AN and eating disorder not otherwise specified (EDNOS) are shown below.

A total of 77 women were diagnosed as bulimics, of which 46 women fulfilled the criteria for BN (42 of purging and 4 of non-purging subtype) and 31 had EDNOS with bulimia as the predominant symptom but with a frequency of binge eating and compensatory behavior less than twice a week or for a duration of less than three months. In the EDNOS group, the median (range) frequency of binge eating was 1 (1-3), vomiting 1 (0-6), dieting 3 (0-6), exercise 3 (0-6), misuse of laxatives 0 (0-1) and diuretics 0 (0-1) days per week. Sixty women without present or previous eating disorder were selected as controls.

Detailed gynecological history including information about the pattern of menstrual periods was obtained from the women. Menstrual status was defined as: regular monthly periods (intervals between 24 and 32 days), oligomenorrhea (periods at an interval exceeding six weeks) and amenorrhea (no bleeding for the last three months).

Bulimia nervosa

- Recurrent episodes of binge eating characterized by eating in a discrete period of time an amount of food that is definitely larger than most people would eat and a sense of lack of control over eating
- Recurrent inappropriate compensatory behavior in order to prevent weight gain, such as self-induced vomiting, misuse of laxatives, diuretics or other medications, fasting, or excessive exercise
- The binge eating and inappropriate compensatory behaviors both occur, on average, at least twice a week for three months
- Self-evaluation is unduly influenced by body shape and weight
- The disturbance does not occur exclusively during episodes of AN

Subtypes

Purging type: The person regularly engages in self-induced vomiting or the misuse of laxatives, diuretics or enemas

Non-purging type: The person uses other inappropriate compensatory behaviours, such as fasting or excessive exercise

(*APA 1994*)

Anorexia nervosa

- Refusal to maintain body weight at or above a minimally normal weight for age and height (e.g. body weight less than 85% of that expected or BMI < 17.5)
- Intense fear of gaining weight or becoming fat, even though underweight
- Disturbance in the way in which one´s body weight or shape is experienced
- Amenorrhea, i.e. the absence of at least three consecutive menstrual cycles

Subtypes

Restricting type: The person has not regularly engaged in binge-eating or purging behaviour

Binge eating/purging type: The person has regularly engaged in binge-eating or purging behaviour

(*APA 1994*)

Eating disorder not otherwise specified

- All of the criteria for AN are met except that the individual has regular menses
- All of the criteria for AN are met except that, despite significant weight loss, the individual´s current weight is in the normal range
- All of the criteria for BN are met except that the binge-eating and inappropriate compensatory mechanisms occur at a frequency of less than twice a week or for a duration of less than three months
- The regular use of inappropriate compensatory behavior by an individual of normal body weight after eating small amounts of food
- Repeatedly chewing and spitting out, but not swallowing, large amounts of food
- Binge-eating disorder: recurrent episodes of binge-eating in the absence of the regular use of inappropriate compensatory behaviors characteristic of BN

(*APA 1994*)

For the clinical material in paper IV general inclusion and exclusion criteria were as for papers I – III. Additional criteria for bulimics and controls were nulliparity, no use of tobacco and not being at risk for thromboembolic disease. Furthermore, all patients should fulfill the criteria for the diagnosis BN of the purging sub-type. The recruitment procedure was as for papers I-III, resulting in a final clinical material of 21 patients and 17 age- and BMI-matched controls.

3.1.1 Ethical considerations

The nature, purpose, and potential risks of the studies were explained to all women and written informed consent was obtained. The local committee for medical ethics approved the study protocols (97-164, 03-286).

3.2 EXPERIMENTAL DESIGN (I-III)

Bulimics and controls were attending the Women's Health Clinical Research Unit, Department of Obstetrics and Gynecology, Karolinska University Hospital at 07.30 in the morning after an overnight fast. Menstruating subjects were investigated in the early follicular phase of the menstrual cycle (cycle days 1-5), while amenorrheic women were investigated on an arbitrary day. Body weight, height and blood pressure were measured in a standardized manner and a pregnancy test was carried out before examination of BMD (see below). A fasting blood sample was collected at 08.00 am from a peripheral vein in a resting state. Serum for biochemical assays was separated after centrifugation and stored at – 70°C, pending analysis. Whole blood for DNA analysis was sent refrigerated to the collaborating research laboratory.

3.2.1 Polycystic ovaries

Gynecological examination including transvaginal ultrasound was performed by the same investigator (LG) using an Acuson Aspen ultrasound equipment with a 7.0 MHz vaginal probe. The ovarian variables assessed were maximum number of follicles in one plane and ovarian volume calculated with the formula $\pi/6$ xD1 x D2 x D3, where D1, D2, and D3 are the three maximum ovarian diameters. Criteria for PCO were defined as at least one ovary exhibiting 12 or more follicles in one plane in combination with an ovarian volume > 10 mL. The diagnostic criteria for PCO syndrome according to Rotterdam Consensus (*Rotterdam Consensus 2004*) are presented below.

Diagnostic criteria for PCOS

1. Oligo- or anovulation
2. Clinical and/or biochemical signs of hyperandrogenism
3. PCO on ultrasound

Two out of three criteria necessary for diagnosis

(*Rotterdam Consensus 2004*)

3.2.2 Hirsutism

Evaluation of body hair growth was assessed by the same investigator (SN) using the Ferriman Gallwey scoring at different body sites (*Ferriman & Gallwey 1961*) as illustrated in Figure 3. A score of 8 or more was defined as hirsutism.

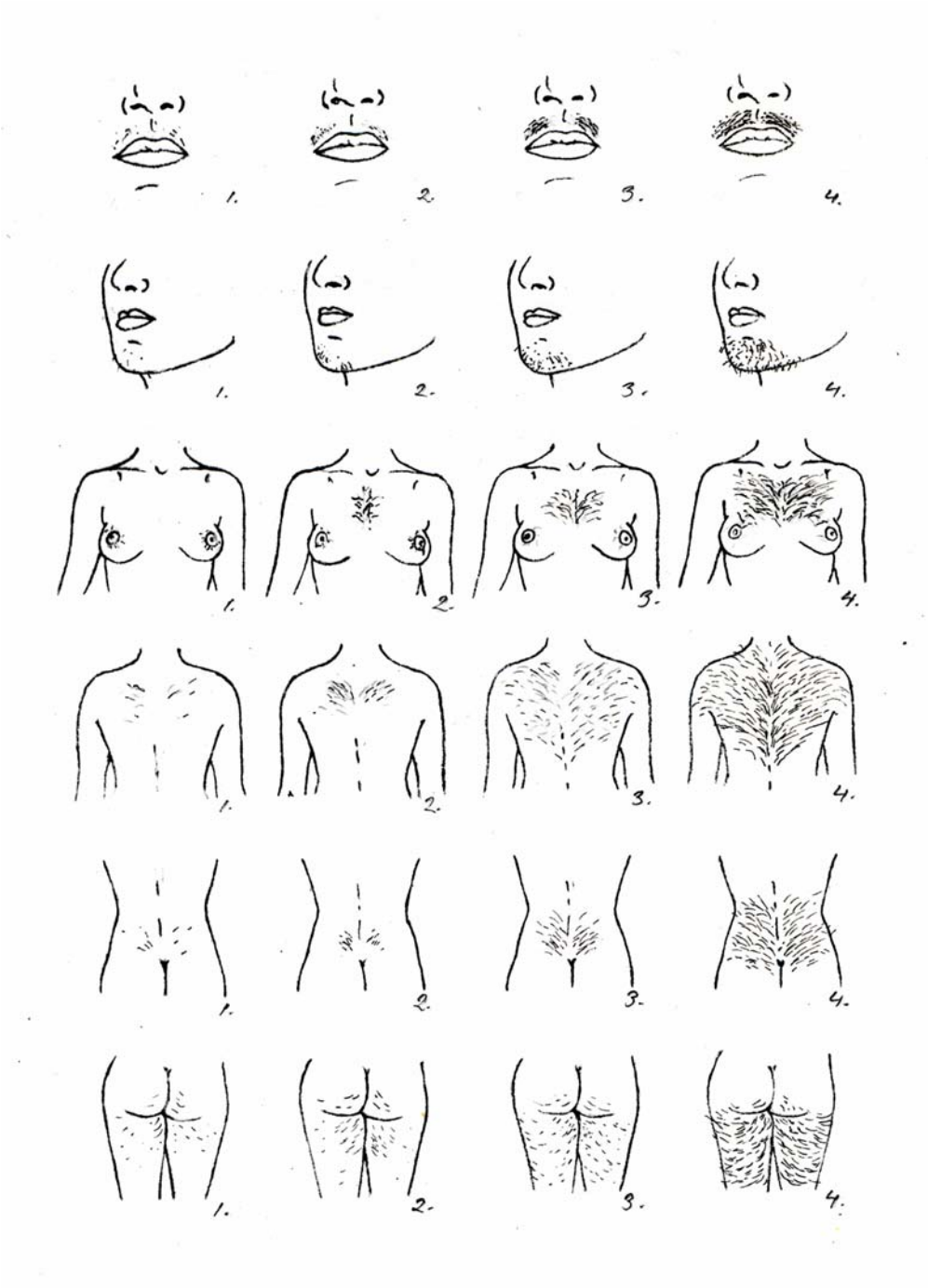

Figure 3. Semiquantitative evaluation of hirsutism in premenopausal women. Each of six body areas is graded separately from no hirsutism (grade 0) to marked hirsutism (grade 4). The grades of all areas are added. A score of 8 or more is defined as hirsutism (*Ferriman & Gallwey 1961*).

3.2.3 Acne

Acne was graded by the same investigator (SN) using a standard method (*Göransson et al. 1978*) (Table 1). A score representing the total "acne load" was obtained by multiplying the number of lesions of each type by the severity index.

Table 1. Definitions of type of acne lesions and their severity index (*Göransson et al. 1978*).

Index	Definition
1/2	Non-inflamed comedones, open and closed (no erythema)
1	Comedones with surrounding erythema, superficial pustules in which the visible pus has a diameter of 2 mm at the most and with no or little erythema
2	Pustules with a diameter exceeding 2 mm or pustules with a significant erythema
3	Deep infiltrates with or without pustules: isolated cysts

3.2.4 Bone mineral density

Determination of BMD was done by dual-energy X-ray absorptiometry (DXA) using a Hologic QDR®4500A scanner (Hologic, Inc., Bedford, MA, USA) (Figure 4). The reproducibility of whole body BMD is calculated as <0.01 g/cm^2 or 0.1 SD (*Nuti et al. 1991; Brismar & Ringertz 1996*). The examination was a whole-body scan. In this scan, the lumbar spine (L1-L4) and both legs were interactively defined and the regional BMD assessed using the standard software. Mean BMD-values of both legs were calculated since the area differences were negligible.

The prevalence of low BMD of the total body was defined as osteopenia if the T-score was between –1 and –2.5 SD of that for young adults (peak bone mass), and osteoporosis if the T-score was below –2.5 SD, according to the World Health Organization (WHO), and as a Z-score below -2 SD of the age-matched mean according to the International Society for Clinical Densitometry (ISDC) guidelines for defining low BMD in premenopausal women (*Writing group for the ISCD Position*

Development Conference 2004). The T- and Z-scores were estimated using mean BMD and SD values supplied by the Hologic equipment manufacturer.

Figure 4. BMD was determined by dual-energy X-ray absorptiometry (DXA) using a Hologic QDR®4500A scanner.

3.3 EXPERIMENTAL DESIGN (IV)

Women with BN and controls were examined before and after treatment with 30 µg of ethinyl estradiol and 3 mg of drosperinone (Yasmin®, Schering AG, Bergkamen, Germany) during three weeks followed by one week of a hormone- and tablet-free interval for a period of three months. Subjects were attending the Women's Health Clinical Research Unit at 07.30 in the morning after an overnight fast. Before treatment, menstruating subjects were investigated in the early follicular phase of the menstrual cycle and amenorrheic subjects on an arbitrary day. At the end of three months of treatment, investigations were performed during the last week of the OC treatment cycle. Measurement of body weight, height, blood pressure, pregnancy test and evaluation of hirsutism and acne were carried out as stated above.

3.3.1 Standardized meal test

An indwelling catheter was inserted into a forearm vein to collect nine blood samples during the three-hour course of the experiment. After two fasting blood samples (15 minutes apart), the subjects were fed a standardized breakfast meal consisting of two sandwiches with cheese, a cup of tea or coffee without sugar and a plate of sour milk with a cup of cornflakes, equivalent to 500 kcal (protein 18%, carbohydrates 54%, fat 28%) (Figure 5). The meal was ingested within 15 min. Blood samples were drawn 15, 30, 45, 60, 90, 120 and 150 min after the start of eating. Serum was separated after centrifugation and stored at -70° C until analyzed. Immediately after the collection of each blood sample, self-ratings for hunger, satiety, gastric distension, nausea and craving for sweets and fat were performed using a visual – analogue scale (VAS) with a range from 0 to 10 for each item (0 = not at all to 10 = extremely).

Figure 5. Standardized meal test with venous blood sampling at specific time points.

3.3.2 Bulimic behavior

The degree of bulimic behavior was evaluated by the same trained research psychologist (VR). The evaluation was based on frequencies of binge eating and compensatory behavior/week, the psychiatric status rating scale (PSR) for BN (*Herzog et al. 1993*) (Table 2)**,** and weight phobia scoring 0-10 (*Gonzalez & Vitousek 2004*). The frequency of binge eating and compensatory behavior was classified into the following four categories: 0, 1, 2-4 and 5-7 days of bulimic behavior per week (*Clinton & Glant 1992*). In the statistical calculations the means were used, i.e. 0, 1, 3 and 6.

Table 2. Psychiatric Status Rating Scale (PSR) for BN (*Hertzog et al. 1993*).

Code	Term	Definition
6	Definite criteria Severe	Meets DSM-IV criteria for BN, resulting in major disruption in functioning (e.g., stays home from work to binge or medical problems develop due to binging and purging)
5	Definite criteria	Meets DSM-IV criteria for BN but has no extreme impairment in functioning
4	Marked	Does not meet DSM-IV criteria for BN but still has obvious symptoms of this disorder (e.g., continues to binge and purge, but less than twice a week)
3	Partial remission	Considerably less psychopathology than full criteria for BN (e.g., only binges once or twice a month and does not purge following a binge)
2	Residual	Although the patient does not meet criteria for BN she still feels she has to fight the impulse to binge
1	Usual self	There is no evidence of BN, and the patient does not fight the urge to binge

3.4 ANALYTICAL METHODS

Serum testosterone, androstenedione (A-4), estradiol-17ß (E2), CCK and ghrelin were determined by radioimmunoassay (RIA) using commercial kits (Table 3). In the assay of A-4 the RIA procedure was preceded by an extra step including extraction with diethyl ether, evaporation and dissolving of the residue in zero calibrator supplied by

the manufacturer of the kit. This step was included in order to avoid possible cross reactions with water-soluble androgen metabolite conjugates.

Serum levels of sex hormone-binding globulin (SHBG), dehydroepiandrosterone sulfate (DHEAS), cortisol, FSH, LH, insulin, IGF-I, thyroid stimulating hormone (TSH), free thyroxin (fT4) and prolactin were determined by direct chemiluminiscence enzyme immunoassay using commercial kits (Table 3). The values of FSH, LH, TSH and prolactin are expressed as IU/L of 2:nd international reference preparation (IRP) FSH 78/549, 1:st IRP LH 68/40, mIU/L of the WHO 2:nd IRP TSH 80/558 and 3:rd WHO IRP prolactin 84/50, respectively.

Serum glucose was determined by the clinical routine method at the Department of Clinical Chemistry, Karolinska University Hospital. The combined metabolic burden of insulin and glucose was described as the fasting insulin resistance index (FIRI), which is the product of insulin and glucose concentrations divided by 25 (*Duncan et al. 1995; Cleland et al. 1996*).

Apparent concentrations of free testosterone were calculated from values for total testosterone, SHBG and a fixed albumin concentration of 40 g/L by successive approximation using a computer program based upon an equation system derived from the law of mass action (*Södergård et al. 1982*). Since the ratio between total testosterone and SHBG (testosterone/SHBG-ratio, “free androgen index”) is considered as a useful index of biologically active testosterone (*Rotterdam Consensus 2004*), this marker was also included in the calculations. The upper reference limit for the testosterone/SHBG at our department is 0.050. Testosterone/SHBG ratios > 0.050 and/or LH/FSH ratios > 2 were classified as biochemical hyperandrogenism. Based on reference data supplied by the manufacturer of the assay kits a LH value < 1 IU/L and/or a FSH value < 3 IU/L was considered to indicate hypothalamic inhibition, a prolactin value > 22 ug/L to be hyperprolactinemia, a TSH value > 4 mIU/L primary hypothyroidism and fT4 value < 11 pmol/L combined with a TSH value within the normal range relative hypothyroidism.

Table 3. Methods, manufacturers, detection limits and within and between assay coefficients of variation (CV) for the different hormones and binding protein. RIA = radioimmunoassay, CEIA = chemoluminiscence enzyme immunoassay.

Analyte	Method	Manufacturer	Detection limit	Within assay CV	Between assay CV
Testosterone	RIA	Diagn. Products Corp.	0.1 nmol/L	6 %	10 %
A-4	RIA	Diagn. Products Corp	0.14 nmol/L	5.7 %	8.4 %
E2	RIA	Orion Ab	5 pmol/L	3 %	6 %
CCK	RIA	Euro-Diagnostica	0.3 pmol/L	3.8 %	8.9 %
Ghrelin	RIA	Phoenix Pharm Inc.	80 ng/L	5.4 %	9.2 %
SHBG	CEIA	Diagn. Products Corp.	0.2 nmol/L	6.5 %	8.7 %
DHEAS	CEIA	Diagn. Products Corp.	0.8 µmol/L	8.2 %	12 %
Cortisol	CEIA	Diagn. Products Corp.	0.2 nmol/L	6.5 %	8.7 %
FSH	CEIA	Diagn. Products Corp.	0.1 IU/L	8 %	8 %
LH	CEIA	Diagn. Products Corp.	0.7 IU/L	6 %	9 %
Insulin	CEIA	Diagn. Products Corp.	2 mIU/L	4.3 %	5.4 %
IGF-I	CEIA	Diagn. Products Corp.	20 µg/L	3.6 %	6.6 %
TSH	CEIA	Diagn. Products Corp.	0.002 mIU/L	5 %	10 %
fT4	CEIA	Diagn. Products Corp.	1.9 pmol/L	4.5 %	5.6 %
Prolactin	CEIA	Diagn. Products Corp.	0.2 µg/L	2.6 %	7.2 %

3.5 MUTATION ANALYSIS (III)

Genomic DNA was prepared using QIAamp® DNA MiniKit (QIAGEN). All polymerase chain reaction (PCR) amplifications were performed using AmpliTaq Gold (Roche) according to standard protocols. All primers were designed to cover intron-exon borders of the ERβ gene. Three ERβ single nucleotide polymorphisms (SNPs) were studied: 1082G→A, 1730 G→A and ERβ cx +56 G→A. Restriction enzyme assays were performed according to standard procedures.

Mutation analysis was undertaken using temperature-modulated heteroduplex analysis (TMHA) on an automated high performance liquid chromatography (HPLC)

instrument. Heteroduplex and homoduplex generations were performed by heating the PCR products. Each PCR product was analyzed using the WAVE® system (Transgenomic). Exons with an abnormal TMHA profile were sequenced and compared with published genomic sequence of the ERβ gene. Amplified products were purified using QIAquick® PCR purification kit (QIAGEN). Sequencing reactions were performed using a BigDyeTM Terminator Cycle Sequencing kit (Applied Biosystems). Samples were resolved in an ABI377 automatic sequencer (Applied Biosystems).

Human ERβ 530 cDNA cloned into the *EcoR*I sites of the pSG5 expression vector (Stratagene, La Jolla, CA) was used as template for mutagenesis. Mutant construct (pSG5-hERβ 530 R221G) was produced by introduction of a 1 bp substitution (A→G) using the QuickChange XL site-directed mutagenesis kit (Stratagene). The substitution was confirmed by DNA sequencing. HEK 293 cells were cultured and plated in 24-well plates 24 h prior to transfection. Transfections using the Superfect reagent (QIAGEN) were performed according to the manufacturer's protocol. ERE-TK-LUC was co-transfected with pSG5-hERβ 530 or pSG5-human ERβ 530 R221G. A pRL-TK control plasmid, containing the *Renilla* luciferase gene, was used as an internal control. The pSG5 vector was used to equalize plasmid concentrations. Upon transfection, the medium was changed and hormone or vehicle was added simultaneously. Cells were harvested 24 h after transfection and luciferase activities were determined.

3.5.1 Analysis of the CA repeat polymorphism of the ERβ gene

Recent data suggest that a polymorphic dinucleotide cysteine/adenine (CA) repeat of the ERβ gene may be related to androgen levels in women (*Westberg et al. 2001*). We therefore also studied the CA repeat polymorphism of the ERβ gene in bulimics and controls.

Genotyping was performed using PCR with fluorescently labeled primers (Amersham Biosciences), followed by capillary electrophoresis on a MegaBACE 1000 instrument (Amersham Biosciences). The PCR was performed using 2.5 ng of genomic DNA in a total volume of 5µl (2.5 mM $MgCl_2$, 0.25 mM dNTP, 0.2U Taq, 0.2 µM primers).

Subsequently, 35 cycles of PCR were performed (95°C for 30s, 58°C for 30s, 72°C for 30s). Forward primer sequence: CATGGTCTGTACCCAGGTG (5´labelled with FAM); Reverse primer sequence: TGAATGAGTGGGCCTCC.

The reactions are cleaned by column filtration using Sephadex G-50 (superfine) and size standard (ET-400R) is added. Sample injection is electrokinetic and after 5 minutes pre-run (10 kV) samples are run (9kV) for 60-75 minutes at 44°C. Allele calling was done using the Genetic Profiler v 2.0 software (Molecular Dynamics) and two independent scorers read all results. Data was checked for Mendelian errors with PEDCHECK software. If discrepancies were found between the files, the PCR and analysis was repeated.

3.6 STATISTICS

Normally distributed data are presented as arithmetic means and SDs or 95% confidence intervals, otherwise as median and range. Some of the variables were log-transformed before the formal analyses since the distribution was positively skewed (II). Comparisons between two groups were performed with t-test for independent samples or Mann-Whitney U-test according to distribution. Comparisons between three groups or more were performed by Kruskal-Wallis test followed by *post-hoc* analysis with t-test for independent samples or Mann-Whitney U-test (I). The chi-square test and the Fisher's exact test were used to analyze variables measured on a nominal scale. When comparing hormone variables between groups, statistical analyses were adjusted for smoking using two-way ANOVA or stratified Mann-Whitney U test (I). DXA values in subgroups of bulimics were compared with those in controls by statistical analysis adjusted for BMI using ANCOVA (II). Effects of OC treatment were tested by t-test for paired observations or by Wilcoxons signed rank test according to distribution (IV). Correlations were performed with Spearman rank correlation test or by linear regression when appropriate (I, IV) or with Pearson´s product moment correlation coefficient (II). Forward stepwise multiple linear regression analysis was used to evaluate to what extent the variation in spinal BMD could be explained by endocrine and nutrition-related variables (II). For the stepwise selection we used a criterion for entry of a p-value < 0.10 and for removal of a p-value > 0.10. The significance level was set at $p < 0.05$.

4 RESULTS

4.1 MENSTRUAL DISTURBANCES AND HYPERANDROGENIC SYMPTOMS (I, IV)

Menstrual disturbances were much more common among women with bulimia than in healthy controls. In the clinical material of 77 bulimic women (I) 31.2% had a menstrual disorder, of which 23.4% was diagnosed as oligomenorrhea and 7.8% as amenorrhea. In comparison, oligomenorrhea was found in 1.7% of the 59 control women and none had amenorrhea. The duration of present menstrual disorder showed a highly significant positive correlation with duration of bulimia. Among the 77 bulimics, 51.9% had a history of amenorrhea (including those with present amenorrhea), whereas none in the control group had been amenorrheic. In paper IV, six out of 21 (28.6%) women with BN had menstrual disturbances (4 oligomenorrhea, 2 amenorrhea), whereas all 17 controls had regular menstruation.

4.1.1 Endocrine results

The total group of women with bulimia and the subgroup of bulimics with menstrual disturbances had significantly higher value for the testosterone/SHBG-ratio than controls (I). Furthermore, levels of free testosterone were increased among oligo-/amenorrheic bulimics. FSH and LH levels were lower in bulimics and oligo-/amenorrheic bulimics had the lowest FSH levels, differing significantly from controls. However, there was no difference in the LH/FSH-ratio between groups. Bulimic women had markedly lower levels of E2 and fT4 and clearly higher cortisol levels compared to controls. There were no significant differences between groups in total testosterone, SHBG, DHEAS, A-4, IGF-1, TSH and insulin levels, whereas clearly higher glucose levels were found in the bulimics. In paper IV, the women with BN had significantly higher total levels of testosterone than controls.

Endocrine disorders in the 24 bulimic women with menstrual disturbances (I) are shown in Table 4. Seven (29.2%) of the bulimic women with menstrual disturbances had biochemical hyperandrogenism. Hypothalamic amenorrhea was diagnosed in six women (25%) and relative hypothyroidism in four women (16.7%). Primary hypothyroidism was diagnosed in one women and hyperprolactinemia in another woman. In five oligo-/amenorrheic bulimics no specific endocrine disorder was found.

Table 4. Endocrine disorders in bulimic women with menstrual disturbances (I).

n total 24	Endocrine disorder
7	Hyperandrogenism (testosterone/SHBG ratio > 0.050 and/or LH/FSH ratio > 2)
6	Hypothalamic amenorrhea (LH value < 1 IU/L and/or FSH value < 3 IU/L)
4	Relative hypothyroidism (fT4 value < 11 pmol/L combined with a TSH value within the normal range)
1	Primary hypothyroidism (TSH value > 4 mIU/L and a fT4 value < 11 pmol/L)
1	Hyperprolactinemia (prolactin value > 22 μg/L)
5	Idiopathic (no specific endocrine disorder was detected)

4.1.2 Hirsutism and acne

Hirsutism score was clearly and significantly increased in bulimic women compared to controls, as was the frequency of pathologically increased hirsutism (I). The hirsutism score in the bulimics was not influenced by menstrual status but by hormone levels. Hirsutism score and indices of biologically active testosterone were positively correlated in bulimics but not in controls, while there were no major differences in serum androgens between groups. There were also strong positive correlations between hirsutism score and the free testosterone/E2-ratio in the bulimics (Figure 6). There were no differences in acne score between bulimics and controls (I, IV).

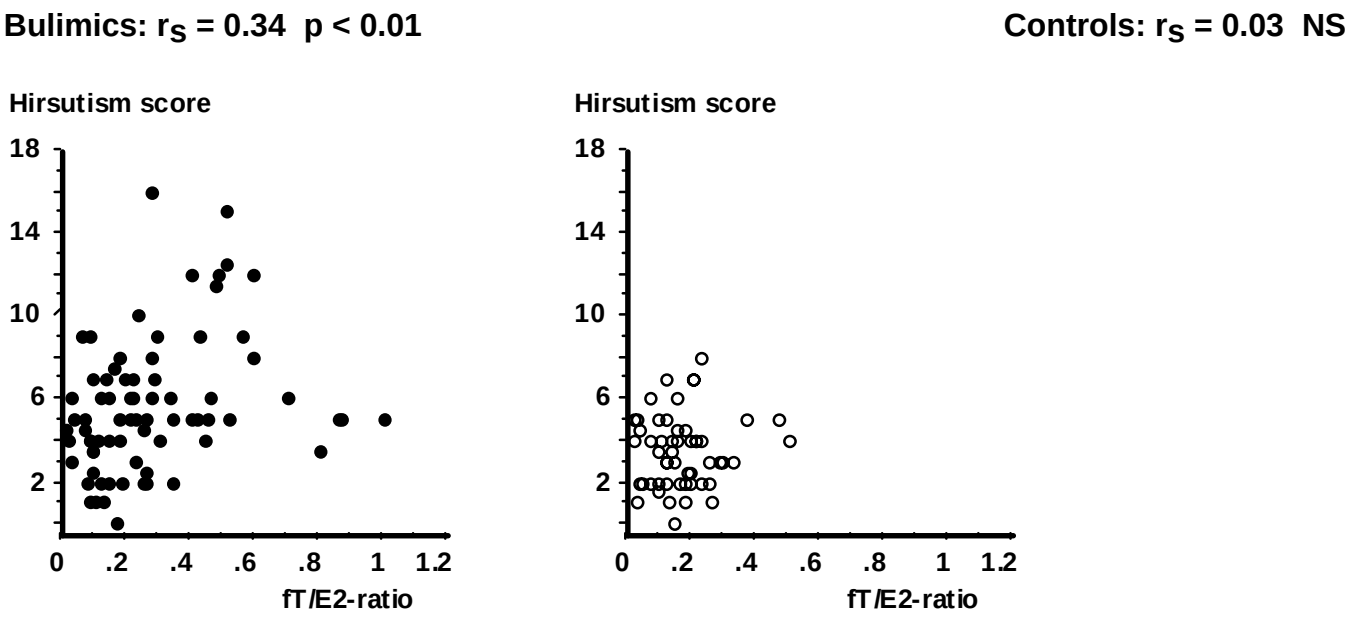

Figure 6. Correlation between hirsutism score and the free testosterone/E2-ratio in bulimics and controls. NS = not significant.

4.1.3 Occurrence of PCO and PCOS (I)

The frequency of the three criteria of which two shall be present for a PCOS diagnosis is given in Table 5. The diagnosis of PCOS was significantly more common among bulimics than among controls.

Table 5. Frequency of criteria for PCOS diagnosis in bulimics and in controls (*p < 0.05, ***p < 0.001).

Criteria	**Bulimics** n = 77	**Controls** n = 59
1. Oligo- or anovulation (%)	31.2***	1.7
2. Clinical and/or biochemical hyperandrogenism (%)	27.3*	11.9
3. PCO on ultrasound (%)	7.1	6.8
PCOS (%)	16.6*	1.7

4.2 BONE MINERAL DENSITY (II)

Bulimics had significantly lower spinal BMD and higher frequency of osteopenia in the total body than controls. Osteoporosis and a Z-score below - 2 were found in two bulimics but in no control. BMD was not associated with type of diagnosis (BN or EDNOS). There was a clearly higher frequency of smokers in the bulimics compared to the controls. However, smoking was not found to be associated with menstrual status, hormone values or BMD.

Subgroups of bulimics with a history of amenorrhea (51.9%) or previous AN (23.4%) had significantly lower total and spinal BMD than controls, whereas subgroups without these conditions had BMD values comparable with controls. Endocrine factors independently associated with BMD in bulimics were a history of amenorrhea, cortisol and testosterone levels. Significant nutrition-related factors were present BMI and history of AN. Minimum BMI, reflecting the degree of previous AN, was also clearly associated with BMD in bulimics (Figure 7).

Multiple regression analysis including all independent significant variables revealed previous AN to be the strongest determinant of spinal BMD in bulimics, accounting for 34% of the variance, while associations between endocrine factors and BMI disappeared. Endocrine factors correlated with previous AN and BMI.

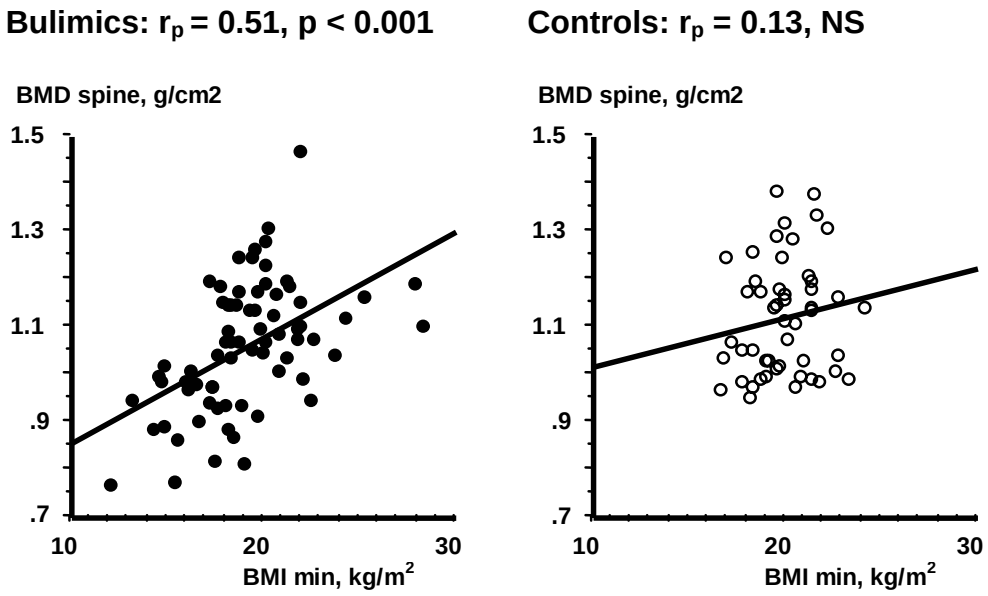

Figure 7. Correlation between spinal BMD and minimum BMI in bulimics and controls. NS = not significant

4.3 ER-ß GENE POLYMORPHISMS (III)

Two common polymorphisms of the ERß gene (1730 G→A and ERβ cx +56 G→A) were found to be associated with bulimia (Table 6). Both BN and ENDOS were independently associated with the ERß polymorphisms, whereas menstrual disturbances were not independently associated with these polymorphisms.

Table 6. Allele frequencies in 76 bulimic patients and 60 controls.

Polymorphism	Study group	Variant allele frequency	p-value	OR
1082 G→A	Bulimics Controls	4.6% 1.7%	ns	2.85
1730 G→A	Bulimics Controls	43% 26%	0.003	2.20
ERβ cx +56 G→A	Bulimics Controls	61% 43%	0.003	2.06

OR = odds ratio, ns = not significant

Estimated haplotypes and associated relative risk are shown in Table 7. Haplotype analysis supports that both studied ERβ polymorphisms are associated with increased risk of disease independently.

Table 7. Estimation of haplotypes (1730 G→A and ERβ cx +56 G→A) in 76 bulimic patients and 60 controls.

Haplotype	Bulimics %	Controls %	OR
GG (wild-type)	37	56	-
GA	20	18	1.6
AA	41	25	2.5
AG	2	1	3.5

OR = odds ratio

4.3.1 Identification of novel variants of the ERβ gene

We screened all exons of the ERβ gene in the bulimic patients and controls for novel variants. Two new and rare intron variants were identified (ERβ 8 -4 A→G and ERβ 8 -68 C→T). However, they do not affect the primary structure of the ERβ protein and occurred in both bulimics and controls.

In one bulimic patient, a 661 A→G change was identified, which changes the primary structure of the ERβ protein. This mutant receptor was analyzed in a transient transfection system. However, this assay revealed no differences between the ERβ wild type and ERβ R221G proteins with regard to transcriptional activation.

4.3.2 The CA repeat polymorphism of the ERβ gene and androgen levels

The CA repeats ranged from 11 to 33 CAs with a median length and cut-off limit of 18. Alleles were divided into subgroups of short repeats (18 or fewer), and long repeats (19 or more). The subjects could thus be divided into three groups; those with short alleles (SS); those with two long alleles (LL); and those with one short and one long allele (SL). There was no significant difference in the distribution of CA repeats between bulimics and controls. However, comparisons of the SS, LL and SL subgroups in the combined material of bulimics and controls revealed a significant difference with respect to levels of free testosterone (Figure 8).

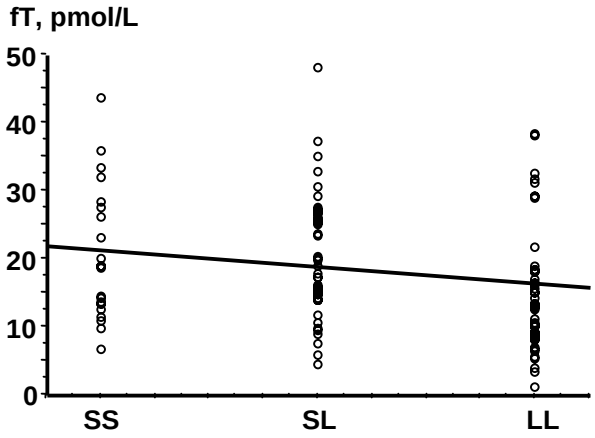

Figure 8. Levels of free testosterone in bulimics and controls carrying the SS, SL and LL variants of the ERβ gene, respectively (r_p = -0.18, $p < 0.05$).

4.4 EFFECTS OF ANTIANDROGENIC TREATMENT ON APPETITE AND EATING BEHAVIOR (IV)

Before treatment, bulimics had significantly higher meal-related responses for nausea and craving for sweets and fat, and clearly lower postprandial release of CCK than controls. However, the decrease in ghrelin levels in response to the meal was comparable between groups.

Treatment with an antiandrogenic OC significantly reduced meal-related hunger and gastric distention and tended to decrease craving for sweets in bulimics. In contrast, OC treatment increased craving for fat in the controls. Furthermore, OCs caused a suppression of postprandial CCK release in the controls, whereas CCK secretion was unchanged by OC treatment in the bulimics. Ghrelin secretion did not change in response to OC treatment and was comparable between groups.

OC treatment improved bulimic behavior by a significant reduction in the frequency of compensatory behavior, which was related to a decrease in testosterone levels (Figure 9). Overall, six out of 21 bulimics displayed reduced bulimic symptoms. This subgroup of bulimics had significantly higher pretreatment levels of testosterone than the other group of bulimics. Furthermore, the responders had higher frequency of binge eating and compensatory behavior at baseline than the non-responders.

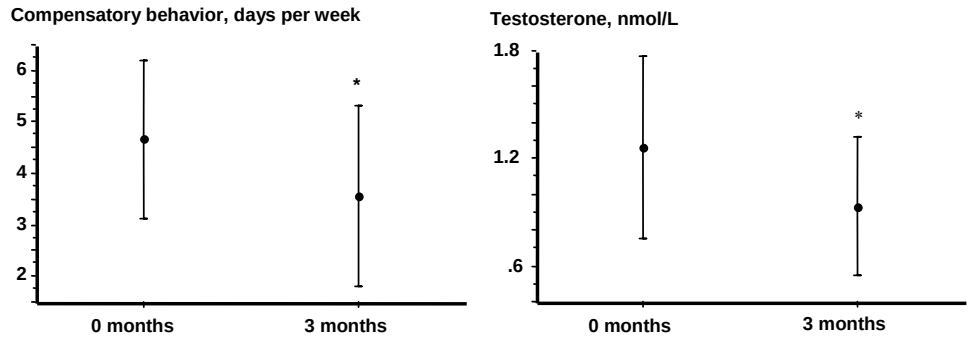

Figure 9. The frequency of compensatory behavior and testosterone levels in 21 bulimics before and during treatment with an antiandrogenic OC. Values are mean ± SD, (*$p < 0.05$).

5 DISCUSSION

5.1 MENSTRUAL DISTURBANCES AND PCOS IN BULIMIC WOMEN

In agreement with previous studies we found a high frequency of menstrual disturbances in bulimic women (*Gendall et al. 2000; Crow et al. 2002*). The mechanisms of oligo-/amenorrhea in these women have been unclear. We identified hyperandrogenism as a common endocrine characteristic in bulimic women with menstrual disturbances. This finding is also supported by previous reports showing elevated androgen levels and increased symptoms of hyperandrogenism like PCOS in bulimic women (*Gupta et al. 1992; McSheery 1992; Sundblad et al. 1994; Cotrufo et al. 2000; Monteleone et al. 2001*). However, some endocrine changes associated with PCOS, such as insulin resistance, hyperinsulinemia and an increased LH/FSH-ratio, were not present in our oligo-/amenorrheic bulimics. Instead, there were bulimics displaying a hormonal profile in accordance with hypothalamic inhibition of the reproductive system, including very low levels of FSH, LH and E2 and increased levels of cortisol. Furthermore, some bulimics with menstrual disturbance had low levels of fT4 combined with normal TSH levels. Low levels of gonadotropins and of fT4 have been reported previously in bulimics (*Schweiger et al. 1992; Cotrufo et al. 2000; Resch et al. 2004*). These endocrine disorders are similar to those associated with AN and most probably reflect relative energy deficiency due to periodical starvation. Thus, it seems that different mechanisms may explain menstrual disturbances in bulimic women, and hyperandrogenism and hypothalamic oligo-/amenorrhea seem to be the most important ones.

The apparent increase of hirsutism in the bulimic women is a novel finding of the present work. Still serum androgen markers like the testosterone/SHBG-ratio and free testosterone levels were only slightly increased compared to controls and adrenal androgen levels were normal. Significant correlations between hirsutism index and androgen markers were found in the bulimics but not in the controls, indicating a role of androgen status for hirsutism in the women with bulimia. One may speculate about an increased androgen sensitivity in bulimic women, either due to an increased 5α-reductase activity in the hair follicles, leading to an increased local production of the terminal biologically active androgen dihydrotestosterone (*Azziz et al. 2000*) or an

increased androgen receptor density. Estrogens are known to inhibit 5α -reductase activity in human dermal papillae (*Niiyama et al. 2001*). We found strong positive correlations between hirsutism score and the free tesosterone/E2-ratio in the total group of bulimics, as well as, in bulimics with menstrual disturbance. When compared to controls, the testosterone/SHBG-ratio and free testosterone levels in the bulimics were both elevated by 20% but the free tesosterone/E2-ratios by 48%. An imbalance in estrogen/androgen activity has been suggested to be of importance for the development of hirsutism (*Tulchinsky & Chopra 1974*) and may thus play a role for hirsutism in bulimic women.

In contrast to some previous studies (*McCluskey et al. 1992; Jahanfar et al. 1995; Raphael et al. 1995*), we found no increased frequency of PCO in bulimics, not even in the oligo-/amenorrheic women. However, in the above mentioned studies, ultrasound was performed transabdominally and PCO was defined according to Adams and colleagues, i.e. at least 10 follicles between 2 and 8 mm in diameter in one plane arranged around an increased amount of stroma (*Adams et al. 1986*). When we recalculated the occurrence of PCO using the definition by Adams and colleagues, we found a frequency of 11.7 % among the bulimics and 10.2 % in the controls (not significant). In agreement with our findings, Michelmore and coworkers (*2001*) failed to demonstrate a significant association between PCO and binge eating. Although androgen excess is often associated with PCO ultrasound morphology this is not mandatory (*Azziz 2005*), and also many women with nonandrogenic disorders may demonstrate PCO on ultrasound (*Hassan & Killic 2003*). According to the new diagnostic criteria of PCOS (*Rotterdam consensus 2004*), PCO ultrasound morphology, oligo-/anovulation and signs of hyperandrogenism are the three criteria of which two are necessary for the diagnosis. Based on this definition, the occurrence of PCOS was 16.6 % in the bulimic women and significantly higher compared to 1.7 % in the controls. The prevalence of PCOS in the general population according to the new diagnostic criteria is to be determined.

What is the etiological connection between bulimia and PCOS? It has been suggested that ultrasound PCO morphology may be secondary to abnormal eating behavior (*Treasure et al. 1988; Morgan et al. 2002*). In support of this, Morgan and co-workers showed that recovery from bulimia in three out of eight patients was associated with

normalization of ovarian morphology in a 9-year follow-up study (*Morgan et al. 2002*). However, ultrasound ovarian morphology was normal in our bulimic women. A more likely explanation for an association between bulimia and PCOS may be that hyperandrogenism or increased androgen sensitivity is the primary condition, which could predispose for the development of bulimic behavior and associated psychiatric comorbidity (*Eriksson et al. 1992; Baisher et al. 1995*). Testosterone is known to stimulate appetite (*Earley & Lleonard 1979*) and high androgen levels in women have been associated with impaired impulse control, irritability and depression (*Eriksson et al. 1992; Baischer et al. 1995*). These symptoms are also common features in women with bulimia (*Bergman & Eriksson 1996; Sundblad et al. 2005*). Furthermore, bulimic women are more sexually experienced and sexually experimental than control women (*Abraham 1998*). Again this may relate to increased androgen activity since androgens have well-known stimulatory effects on female sexuality (*Flöter et al. 1997*).

Our study supports an increased frequency of PCOS in bulimic women. We suggest that a subset of bulimics may have a primary endocrine/metabolic disorder, which could promote bulimic behavior. Androgens are known to stimulate appetite and could also impair impulse control. Hypothalamic inhibition of the reproductive axis during periods of starvation may be another underlying mechanism of menstrual disturbances in women with bulimia. Menstrual disturbances and clinical signs of hyperandrogenism should be evaluated in bulimics in order to provide adequate medical care and treatment.

5.2 INFLUENCE OF ENDOCRINE FACTORS AND PREVIOUS ANOREXIA ON BONE MASS IN BULIMIC WOMEN

The bulimic women in our study had significantly lower spinal BMD and a higher frequency of osteopenia in the total body than controls. This is in accordance with some previous studies (*Howat et al. 1989; Joyce et al. 1990; Newton et al. 1993; Baker et al. 2000*) but not with others (*Newman & Halmi 1989, Sundgot-Borgen et al. 1998; Zipfer et al. 2001*). Subgroups of bulimics with a history of amenorrhea or previous anorexia had the lowest total and spinal BMD, whereas those without such history did not differ from controls. The relative influence of endocrine factors and of previous AN on BMD in bulimic women was investigated.

We found that a history of amenorrhea, cortisol and testosterone levels were significantly associated with spinal BMD in the bulimic group. However, present menstrual disturbance showed no correlation with BMD, whereas E2 levels tended to be positively correlated with spinal BMD. Nutrition-related factors, i.e. BMI and a history of AN displayed stronger associations with total and spinal BMD than endocrine factors in the bulimic women.

When endocrine and nutritional variables were included in a multivariate analysis, a history of AN was by far the strongest predictive factor for bone mass in the bulimics, accounting for 35% of the variance in spinal BMD. All endocrine factors and current BMI were insignificant. This finding again demonstrates the devastating effect of AN on the skeleton (*Legroux-Gerot 2005; Misra & Klibanski 2006*). Despite showing hormonal aberrations when compared to controls, bulimics without previous AN had a normal bone mass. A varying element of past AN in the different study populations of bulimics may explain the divergent results obtained in previous studies (*Howat et al. 1989; Newman & Halmi 1989; Joyce et al. 1990; Newton et al. 1993; Sundgot-Borgen et al. 1998; Baker et al. 2000; Zipfer et al. 2001).*

What is the mechanism for previous AN to cause low bone mass in normal/over-weight bulimic women? Actual bone density in an adult woman is highly dependent on what values for peak bone mass she achieved during her early twenties. Women with a low peak bone mass have a lower reserve to meet subsequent bone loss during the rest of life. Restrictive eating and caloric insufficiency during adolescence will negatively affect bone formation when peak bone mass is attained (*Bonjour et al. 1991*). AN is also associated with amenorrhea that probably contributes to inhibited skeletal development since estrogen deficiency causes increased bone resorption (*Gordon & Nelson 2003*). We found that a history of amenorrhea was strongly correlated to previous AN in the bulimic women. Although recovery from AN can improve BMD (*Legroux-Gerot 2005; Miller et al. 2006*), the consistent influence from a low peak bone mass may explain osteopenia/osteoporosis in bulimic women with previous AN.

Our study shows that bulimia *per se* does not influence bone mass negatively. Only bulimics with a history of AN could be considered a risk group for osteoporosis and non-traumatic fractures later in life. It is therefore important to evaluate BMD in this

subgroup of bulimics in order to take relevant action, such as optimizing nutritional intake and restore hypogonadism, to prevent further bone loss.

5.3 ERß GENE VARIANTS IN BULIMIC WOMEN

Recent studies suggest that there is a substantial genetic influence on eating disorders (*Bulik et al. 2000; Fairburn & Harrison 2003; Gorwood et al. 2003*). In this study we investigated if genetic variations in the ERβ gene could play a role in bulimic disease. The rationale for this approach was based on the much higher occurrence of eating disorders among women than men and that estrogen is known to regulate eating behavior (*Lindén et al. 1990; Andersen 1999; Geary 2001*). Furthermore, the ERβ gene is located on chromosome 14, in a region which has shown linkage with BN (*Bulik et al. 2003*).

We scored three common polymorphisms in the ERβ gene and found that two ERβ SNPs were independently associated with bulimic disease in a Swedish patient cohort. However, a smaller German study did not provide evidence for an association between BN and ERβ polymorphisms (*Rosenkranz et al. 1998*). Further analysis showed that both diagnosis BN and EDNOS were independently associated with the ERβ polymorphisms but not menstrual irregularities. Our clinical observations revealed no distinction between bulimic women with the risk allele and those without. We also detected a heterozygous point mutation in the ERβ hinge region in one bulimic patient. Further analysis of this mutant in a transient transfection assay did not support impaired receptor function. However, this assay only measures a subset of ERβ functions and therefore it is still possible that this mutation may affect the transcriptional potential of the receptor.

Our findings point towards a possible role of genetic variations in the ERβ gene in the etiology of bulimic disease. Whether this role derives from its functions in peripheral and/or central systems will need to be addressed. It remains a possibility that these polymorphisms *per se* contribute to ERβ function and the etiology of disease. As they are located in the 3' UTR they could affect mRNA stability. However, it seems more likely that these polymorphisms are in linkage disequilibrium with a functional change in the ERβ gene or other genes.

Interestingly, the CA repeat polymorphism of the ERβ gene has been associated with androgen and SHBG levels in women. Westberg and co-workers (*2001*) demonstrated that women with relatively short alleles displayed higher levels of testosterone and lower levels of SHBG than women with many CA repeats. We also found that higher levels of free testosterone correlated with shorter CA repeats of the ERβ gene. However, there was no difference in the distribution of genotypes between bulimics and controls. The present data suggest that the ERβ gene influences androgen levels, which in turn may play a role for the development of bulimic behavior.

5.4 EFFECTS OF ANTIANDROGENIC TREATMENT IN BULIMIA NERVOSA

Treatment with an antiandrogenic OC for three months reduced subjective meal-related hunger and gastric distention in women with BN. Meal-related CCK secretion was lower in bulimics before treatment but did not decrease after treatment as in the controls. No differences and changes were found in ghrelin levels. Improved bulimic behavior following treatment was related to reduced testosterone levels.

CCK is well established as a satiety peptide and meal-related CCK secretion correlates to subjective appetite (*Hirschberg et al. 1994; Devlin et al. 1997*). As shown previously (*Devlin et al. 1997*), bulimics had a reduced postprandial CCK release and increased nausea and craving for sweets and fat before treatment compared to controls. Delayed gastric emptying due to binge eating and an enlarged gastric capacity have been put forward as causes of impaired meal-related CCK secretion in bulimics (*Devlin et al. 1997*). Since food in the duodenum stimulates the release of CCK, delayed gastric emptying may lead to reduced postprandial CCK secretion and in turn impaired satiety response, which may maintain bulimic behavior.

In accordance with previous findings (*Karlsson et al. 1992; Hirschberg et al. 1996*), OC treatment suppressed postprandial CCK release in the controls. OC treatment also increased craving for fat in this group of women. Reduced CCK secretion may therefore have a role in increased appetite during OC treatment in the controls although there was no significant correlation between CCK and appetite ratings. In contrast, OC treatment did not affect meal-related CCK secretion in the bulimics. On the other hand,

OC treatment reduced ratings of hunger, gastric distention and craving for sweets, thus improving meal-related appetite response in bulimics.

No differences in ghrelin levels were found between bulimics and controls. The results are in agreement with some previous studies (*Nakazato et al. 2004; Monteleone et al. 2005*), however, other studies have reported elevated secretion in bulimics (*Tanaka et al. 2002; Monteleone et al. 2003*). Ghrelin levels were not affected by OC treatment and do not seem to be of importance for changed appetite by OCs.

Eating behavior, notably compensatory behavior, was improved by OC treatment in the bulimics. The decrease in compensatory behavior was significantly related to decreased testosterone levels following treatment. Furthermore, bulimics who responded with decreased bulimic behavior had higher pretreatment levels of testosterone and higher frequency of binge eating and of compensatory behavior than those who did not. These findings are in line with previous reports on associations between high androgen levels on the one hand and increased appetite, reduced postprandial CCK secretion and a deranged appetite regulation and impaired impulse control on the other (*Sundblad et al.1994; Hirschberg et al. 2004*).

Treatment with the androgen receptor antagonist flutamide has reported to have beneficial effects in bulimics (*Bergman & Eriksson 1996; Sundblad et al. 2005*). However, this drug has been associated with adverse liver effects, which may limit its long-term use. The present pilot study, showed similar beneficial effects of treatment with an antiandrogenic OC, which is widely used and generally well tolerated with few side-effects (*Foidart 2000; Sitruk-Ware 2005*). The ethinyl estradiol/drospirenone combination is also reported to reduce premenstrual symptoms (PMS) (*Brown et al. 2002*). As in bulimics, increased levels of testosterone have been reported in women with PMS (*Eriksson et al. 1992*). These women also suffer from increased craving for food during the premenstrual phase, where also bulimics may experience aggravations of their symptoms (*Lee & Lee 1992*).

To conclude, treatment with an antiandrogenic OC improved meal-related appetite response in women with BN and reduced bulimic symptoms in relation to decreased testosterone levels. Although this was an open un-controlled study, our results support

the suggestion that androgens play a role in bulimic behavior and therefore warrant further large scale studies on alternative treatment strategies. Antiandrogenic OC treatment may develop into a new management of women with BN, particularly in those with hyperandrogenic symptoms. Hypothetically bulimia may in some cases be a manifestation of a hormonal constitution rather than a primary psychiatric illness.

5.5 A CRITICAL ASSESSMENT AND FUTURE PROSPECTS

Sex steroids and a variety of physical and psychological factors can affect eating behavior and metabolism in a complex interplay. It is difficult to determine the exact role of androgens in this respect. The levels of androgens vary with age, menstrual function, body weight and life-style. Certainly, the data presented in this thesis should be interpreted with caution.

The subjects in this work, both the women with bulimia and the controls, were all recruited after advertisement. The advantage with this procedure was the possibility to recruit bulimics without ongoing treatment. It is well-known that many women with eating disorders do not attend medical care since eating disorders are afflicted with embarrassment, denial, shame, and secrecy (*Vitousek & Stumpf 2005*). On the other hand, the recruitment of control women might have been skewed, since "too healthy" women may have been included. For example, the frequency of menstrual disturbances in this group was very low.

We used a semi-structured clinical interview, the DIAB version 3.0 (*Clinton & Glant 1992*), as a tool for diagnosing BN and EDNOS according to DSM IV. This interview has been widely used as a clinical instrument since many years. However, it has not yet been validated. On the other hand, when evaluating bulimic symptoms in response to antiandrogenic OC treatment, we also used the validated PSR (*Herzog et al. 1993*) and weight phobia scoring instrument (*Gonzalez & Vitousek 2004*). All evaluations were performed by the same highly experienced research psychologist.

One limitation associated with the present investigation is that some data analyzed were self-reported. Data about history of AN, amenorrhea and minimum BMI may therefore be uncertain. Still, strong correlations were recorded between these variables and BMD.

Blood hormone levels were assessed from single morning samples. This could be another limitation of our study since there is a well-recognized circadian rhythmicity and pulsatility in the secretion of several hormones and binding proteins. Furthermore, it is possible that stress in the experimental situation could have affected the levels of some hormones. However, both patients and control subjects underwent a 30 minutes resting period before blood was drawn and the blood sampling was performed in a standardized manner, at the same time in the morning, and during the early follicular phase of the menstrual cycle in menstruating subjects.

Assessment of hyperandrogenic symptoms such as hirsutism and acne is certainly subjective but it was carried out by the same investigator. However, the evaluation was not blinded regarding the subject's group affiliation (bulimic or control woman). Despite this limitation, hirsutism score and serum androgen markers were highly correlated, which speaks against subjective bias.

In study IV, we evaluated the effects of an antiandrogenic OC on meal-related appetite responses and eating behavior in women with BN compared to controls. This work should be regarded as a pilot study and it was uncontrolled and unblinded. A healthy control group was included to evaluate treatment effects as compared to baseline status. Still, we believe that these preliminary data have sufficient interest to justify larger placebo-controlled studies on the effects of antiandrogens in bulimic women.

This thesis has raised several new questions about hormonal changes and metabolic consequences in women with bulimia. Hyperandrogenism and hypothalamic oligo-/amenorrhea seem to be the most important mechanisms behind menstrual disturbances in bulimics. However, in order to further improve our understanding of the mechanisms underlying menstrual dysfunction, future studies would include investigation of diurnal variations of hormones and binding proteins in groups of amenorrheic and oliogmenorrheic bulimic women.

Our knowledge about the role of androgens in bulimic behavior is inadequate. At present, the treatment of choice for BN is CBT and antidepressant medication. However, there are limited data on long-term effectiveness and a subset of patients with bulimia does not respond adequately to this treatment. Clearly there is a need for

additional treatment strategies. There seems to be an association between bulimic behavior and androgen levels but the mechanisms remain to be clarified. The improvement of bulimic behavior by an antiandrogenic OC is intriguing. It could be that antiandrogens may represent a new therapeutic principle for BN. However, in order to establish treatment efficacy, larger placebo-controlled and long-term studies are needed.

Improved insights in the etiology of bulimia may enhance our possibilities for prevention and treatment of the disease. Further research is needed to clarify genetic variations that could possibly explain the association between endocrine status and eating disorders. Variations in ERβ function may be of particular importance in this respect.

6 GENERAL CONCLUSIONS

- An increased frequency of PCOS in bulimic women was demonstrated. A subset of bulimics may have a primary endocrine/metabolic disorder, which may promote bulimic behavior. Hypothalamic inhibition of the reproductive axis due to periodic starvation may be another underlying mechanism of menstrual disturbances in women with bulimia. Menstrual disturbances and clinical signs of hyperandrogenism should be evaluated in bulimics in order to provide adequate medical care and treatment.

- Bulimia *per se* does not influence bone mass negatively. Only bulimics with a history of anorexia nervosa had a decreased BMD. These women should be considered a risk group for osteoporosis and non-traumatic fractures later in life. Thus, it is important to evaluate BMD in this subgroup of bulimics in order to take relevant action for prevention of further bone loss.

- We found an association between two ERβ polymorphisms and bulimic disease, which points towards a possible role of variations in the ERβ gene in the etiology of bulimic disease. In order to establish the significance of ERβ variants for bulimia, our findings need to be confirmed in other populations and larger studies.

- Treatment with an antiandrogenic oral contraceptive significantly improved meal-related appetite response in women with bulimia nervosa and reduced symptoms in relation to decreased testosterone levels. Our results support the notion that androgens play a role in bulimic behavior. Antiandrogens may therefore develop into a new therapeutic approach in women with bulimia nervosa, particularly in those with hyperandrogenic symptoms.

7 ACKNOWLEDGEMENTS

I start in the name of God, most Gracious, most Merciful

and I wish to express my sincere gratitude and deepest appreciation to all who have supported me during the years working on my thesis. In particular I would like to thank:

Angelica Lindén Hirschberg, my supervisor, mentor and dear friend, for introducing me to the fascinating field of endocrinology, for your excellent scientific guidance and for giving me the opportunity to perform science, for your extensive knowledge and sense for perfection, your generosity, for being enthusiastic, encouraging and your tenderness. For lovely times at your house with your family. I am looking forward to more collaboration.

Kjell Carlström, for sharing your great knowledge not only in steroid biochemistry, but in life itself. I had great time being with you. God bless you, for your generosity and being there for me!

Bo von Schoultz, former Head of the Department of Obstetrics and Gynecology, Karolinska University Hospital, for willingness to share your funds of knowledge, for giving me opportunity for research and for all your support and friendship throughout all these years and also inspiring and valuable comments on the manuscripts.

My co-authors, for excellent expert help. I am especially grateful to Maria Nilsson, Karin Dahlman-Wright, Leena Garoff, Rolf Glant, Hans Jacobsson, Ingrid Dahlman and Jan-Åke Gustafsson.

Lennart Nordström, present Head of the Department of Obstetrics and Gynecology, Karolinska University Hospital, for support and kindness and for creating pleasant working conditions.

Måns Edlund, Head of the Gynecologic unit, my friend, for being very generous and supportive, for being considerate and for providing working conditions that enabled work with my thesis.

Isis Amer-Wåhlin, Head of the Obstetric unit, for stimulating talks, being open and providing pleasant working conditions.

Oddvar Bakos, friend, former Head of the Department of Obstetrics and Gynecology, Karolinska University Hospital, for always having time, your great hospitality, your kindness and support!

Barbro Larson, former Head of the Gynecologic unit, for being always supportive and kind.

Gunvor Ekman Ordeberg, former head of the Obstetric unit, for your kindness and considerateness, for being supportive.

The Research staff at Kvinnohälsan, *Lisbeth, Anna, Liselotte, Berit, Siv* and *Lena*, for excellent help in clinical research and all the short moments of fun we had together.

Catharina Karlsson, beautiful, for always looking great while most of us are "walking around" in our green pyjamas, for being always kind and willing to help, for invaluable help with my thesis.

Astrid Häggblad, for her patient and skilful guidance throughout the PhD student´s period with applications and papers.

All the volunteer women who generously participated in the studies.

Vilhelmina Rommel, for friendship and excellent psychiatric evaluation.

Malin Levin and *Anna Lindström* for psychiatric evaluation.

Elisabeth Berg, for skilful statistical assistance.

All the wonderful *staff* I work with every day, making my work so enjoyable. Special thanks to Ingegärd, Eva-Lisa, Mariette, Majken, Kerstin, Anne, Ingela, Barbro, Cathrine, Helena, Raija, Susanne, Lena, Ulla-Britt and furthermore all my other friends at the S-MVC, Delivery ward, C-23, C-14, C-24, and SESAM.

All *the staff* at the DEXA unit, for kindness and skilful work.

Gabriel Fried, for being always kind and having time for discussions.

Birgitta Byström, my co-author, for being a reliable friend, always listening patiently, letting me feel welcome, her willingness to help.

Yvonne,co-author and Eva, *Berit,* at the FoU-lab, for your friendly support during these years, creating a friendly atmosphere at the lab, always being helpful.

Mohammed, for always being extremely friendly, joyful, making me laugh, cooking me tea and willing to help with millions of things.

Giovanna Marchini, for being so friendly, supportive and encouraging.

Björn Johansson, for valuable comments on manuscript and for always being helpful.

Lalitkumar, for being generous, helpful.

Eva, Ing-Marie, "Cissi", Marie, Viola and Sirkka, you lighten up my day. I feel great being with you!

Friends and colleagues at the Department of Obstetric and Gynecology, for making our time together pleasant. Special thanks to *Eva, Lena, Angelique,Ylva, Maria. Katarina, Marie, Gunnel, Magnus, Berit, Magdalena, Estella, Henrik, Rangeen, Angelos, Kjell, Tomas and Allan.*

Annette Aronsson, for always being friendly, supportive.

Åsta Cerin, for being honest, kind-hearted and having always time to comfort.

Gunnar Söderqvist, friend, for nice laughing moments, understanding the irony of life.

Kristina Gemzell Danielsson, for friendship, for always being enthusiastic and keeping my spirits up.

Marianne van Rooijen, for encouragement and support, for taking great interest in my work, but mostly being a wonderful and true friend!

Eva Lundström, dear friend, for her great encouragement and support, for always being encouraging and for all the wonderful times we had in Luleå.

Anette and *Mirtha*, wonderful friends, for being always there, honest, understanding through these years, for fruitful scientific discussions.

Magnus "maydonoz", for your love and support, being a reliable friend, for all wonderful journeys and laughter.

Olle, for still understanding each other after so many years.

My dear brother *Ulf,* being patient, always helpful, taking care of us. Without your help we could not make it.

My dear sister *Hatice* and my dear brother-in-law *Bo* and their son *Anders,* for your endless love and support all these years. Encouraging and make me believe in myself. I am proud to have you. God bless you!

Tord, my best friend, since thirty-five years, for all love and the laughter through these years!

My beloved daughter ***Ida***, you are the Gods blessing to me. Thank you for choosing me to your mom!

****

This study was supported by grants from

- The Council Resource Center for Eating Disorders
- The Swedish Medical Research Council
- Professor Bror Gadelius Foundation
- Karolinska Institutet Research Fund

8 REFERENCES

Abraham S. Problems with weight control during pregnancy. Med J Aust 1989;151:237.

Abraham S. Sexuality and reproduction in bulimia nervosa patients over 10 years. J Psychosom Res 1998;44:491-502.

Adams J, Polson DW, Franks S. Prevalence of polycystic ovaries in women with anovulation and idiopathic hirsutism. Br Med J (Clin Res Ed) 1986;293:355-9.

Altemus M, Hetherington MM, Kennedy B, Licino J, Gold PW. Thyroid function in bulimia nervosa. Psychoneuroendocrinology 1996;21:249-61.

Andersen AE. Gender-related aspects of eating disorders: a guide to practice. J Gend Specif Med 1999;2:47-54.

American Psychiatric Association (APA). Diagnostic and Statistical Manual of Mental Disorders 3rd edition.Washington, D.C. 1980.

American Psychiatric Association (APA). Diagnostic and Statistical Manual of Mental Disorders 4rd edition.Washington, D.C. 1994.

Asarian L, Geary N. Cyclic estradiol treatment phasically potentiates endogenous cholecystokinin's satiating action in ovariectomized rats. Peptides 1999;20:445-50.

Azziz R, Carmina E, Sawaya ME. Idiopathic hirsutism. Endocr Rev 2000;21:347-62.

Azziz R. Diagnostic criteria for polycystic ovary syndrome: a reappraisal. Fertil Steril 2005;83:1343-6.

Bachrach LK, Katzman DK, Litt IF, Guido D, Marcus R. Recovery from osteopenia in adolescent girls with anorexia nervosa. J Clin Endocrinol Metab 1991;72:602-6.

Bailer UF, Kaye WWH. A review of neuropeptide and neuroendocrine dysregulation in anorexia and bulimia nervosa. Curr Drug Targets CNS Neurol Disord 2003;2:53-9.

Baischer W, Koinig G, Hartmann B, Huber J, Langer G. Hypothalamic-pituitary-gonadal axis in depressed premenopausal women: elevated blood testosterone concentrations compared to normal controls. Psychoneuroendocrinology 1995;20:553-9.

Baker D, Roberts R, Towell T. Factors predictive of bone mineral density in eating-disordered women: a longitudinal study. Int J Eat Disord 2000;27:29-35.

Bergman L, Eriksson E. Marked symptom reduction in two women with bulimia nervosa treated with the testosterone receptor antagonist flutamide. Acta Psychiatr Scand 1996;94: 137-9.

Blinder BJ, Cumella EJ, Sanathara VA. Psychiatric comorbidities of female inpatients with eating disorders. Psychosom Med 2006;68:454-62.

Bonjour JP, Theintz G, Buchs B, Slosman D, Rizzoli R. Critical years and stages of puberty for spinal and femoral bone mass accumulation during adolescence. J Clin Endocrinol Metab 1991;73:555-63.

Brismar T, Ringertz H. Effect of bone density of the head on total body DEXA measurements in 100 healthy Swedish women. Acta Radiol 1996;37:101-6.

Brown C, Ling F, Wan J. A new monophasic oral contraceptive containing drospirenone. Effect on premenstrual symptoms. J Reprod Med 2002;47:14-22.

Buczkowska EO. The role of ghrelin in the regulation of energy homeostatis. Endokrynol Diabetol Chor Przemiany Materii Wieku Rozw 2005;11:39-42.

Bulik CM, Sullivan PF, Wade TD, Kendler KS. Twin studies of eating disorders: a review. Int J Eat Disord 2000;27:1-20.

Bulik CM, Sullivan PF, Kendler KS. Genetic and environmental contributions to obesity and binge eating. Int J Eat Disord 2003;33:293-8.

Cantopher TC, Evans JH, Lacey JM. Pearce. Menstrual and ovulatory disturbance in bulimia. Br Med J 1988;297:836-7.

Carey AH, Chan KL, Short F, White D, Williamson R, Franks S. Evidence for a single gene effect causing polycystic ovaries and male pattern baldness. Clin Endocrinol 1993;38:653-8.

Chan JL, Mantzoros CS. Role of leptin in energy-deprivation states: normal human physiology and clinical implications for hypothalamic amenorrhoea and anorexia nervosa. Lancet 2005;366:74-85.

Chapdelaine PA Jr. Bulimia and polycystic ovarian syndrome? Fertil Steril 1991;56:585-6.

Cleland SJ, Petrie JR, Morris AD, Ueda S, Dorrian CA, Conell JMC. FIRI: A fair insulin resistance index? Lancet 1996;347:770.

Clinton DN, Glant R. The eating disorders spectrum of DSM-III-R. Clinical features and psychosocial concomitants of 86 consecutive cases from a Swedish urban catchment area. J Nerv Ment Dis 1992;180:244-50.

Copeland PM, Herzog DB. Menstrual abnormalities in bulimia. In: JI Hudson and HG Pope, Eds. The psychobiology of bulimia nervosa. Washington, D.C., American Psychiatric Press, 1987, pp 29-54.

Copeland PM, Sacks NR, Herzog DB. Longitudinal follow-up of amenorrhea in eating disorders. Psychosom Med 1995;57:121-6.

Cotrufo P, Monteleone P, d´Istria M, Fuschino A, Serino I, Maj M. Aggressive behavioral characteristics and endogenous hormones in women with bulimia nervosa. Neuropsychobiol 2000;42:58-61.

Crow SJ, Thuras P, Keel PK, Mitchell JE. Long-term menstrual and reproductive function in patients with bulimia nervosa. Am J Psych 2002;159:1048-50.

Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weigle DS. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. Diabetes 2001; 50:1714-9.

Devlin MJ, Walsh BT, Guss JL, Kissileff HR, Liddle RA, Petkova E. Postprandial cholecystokinin release and gastric emptying in patients with bulimia nervosa. Am J Clin Nutr 1997;65:114-20.

Di Francesco V, Zamboni M, Dioli A, Zoico E, Mazzali G, Omizzolo F, Bissoli L, Solerte SB, Benini L, Bosello O. Delayed postprandial gastric emptying and impaired gallbladder contraction together with elevated cholecystokinin and peptide YY serum levels sustain satiety and inhibit hunger in healthy elderly persons. J Gerontol A Biol Sci Med Sci 2005; 60:1581-5.

Duncan MH, Singh BM, Wise PH, Carter G, Alaghband-Zadeh J. A simple measure of insulin resistance. Lancet 1995;346:120-1.

Duncan AE, Neuman RJ, Kramer J, Kperman S, Hesselbrock V, Reich T, Bucholz KK. Are there subgroups of bulimia nervosa based on comorbid psychiatric disorders? Int J Eat Disord 2005;37:19-25.

Earley CJ, Leonard BE. Androgens, estrogens and their anti-hormones: effects on body weight and food consumption. Pharmacol Biochem Behav 1979;11:211-4.

Eckel LA, Geary N. Endogenous cholecystokinin's satiating action increases during estrus in female rats. Peptides 1999;20:451-6.

Ehrmann DA. Polycystic ovary syndrome. N Engl J Med 2005;352:1223-36.

Elger W, Beier S, Pollow K, Garfield R, Shi SQ, Hillisch A. Conception and pharmaco- dynamic profile of drospirenone. Steroids 2003;68:891-905.

Eriksson E, Sundblad C, Lisjo P, Modigh K, Andersch B. Serum levels of androgens are higher in women with premenstrual irritability and dysphoria than in controls. Psychoneuroendocrinology 1992;17:195-204.

Eriksson E. Behavioral effects of androgens in women. In: M Steiner, KA Yonkers, E Eriksson, Eds. Mood disorders in women: Martin Dunitz. London 2000, pp 233-46.

Essah PA, Nestler JE. The metabolic syndrome in polycystic ovary syndrome. J Endocrinol Invest 2006;29:270-80.

Fairburn CG, Cooper PJ. Self-induced vomiting and bulimia nervosa: an undetected problem. Br Med J 1982;284:1153-55.

Fairburn CG, Cooper PJ. The clinical features of bulimia nervosa. Br J Psychiatry 1984: 144:238-46.

Fairburn CG, Hope RA. Changes in behavior in dementia: a neglected research area. Br J Psychiatry 1988;152:406-7.

Fairburn CG, Beglin SJ. Studies of the epidemiology of bulimia nervosa. Am J Psychiatry 1990;147:401-8.

Fairburn CG, Doll HA, Welch SL, Hay PJ, Davies BA, O'Connor ME. Risk factors for binge eating disorder: a community-based, case-control study. Arch Gen Psychiatry 1998;55:425-32.

Fairburn CG, Cooper Z, Doll HA, Norman P, O´Connor M. The natural course of bulimia nervosa and binge eating disorder in young women. Arch Gen Psychiatry 2000;57:659-65.

Fairburn CG, Harrison PJ. Eating disorders. Lancet 2003;361:407-16.

Ferriman D, Gallwey JD. Clinical assessment of body hair growth in women. J Clin Endocrinol Metab 1961;21:1440-7.

Fichter MM, Quadflieg N. Twelve-year course and outcome of bulimia nervosa. Psychol Med 2004;34:1395-406

FlöterA, Nathorst-Böös J, Carlström K, von Schoultz B. Androgen status and sexual life in perimenopausal women. Menopause 1997;4:95-100.

Foidart JM. The contraceptive profile of a new oral contraceptive with antimineralocorticoid and antiandrogenic effects. Eur J Contracept Reprod Health Care 2000;5:25-33.

Franks S, Gharani N, Waterworth D, Batty S, White D, Williamson R, McCarthy M. The genetic basis of polycystic ovary syndrome. Hum Reprod 1997;12:2641-8.

Fuhrmann U, Krattenmacher R, Slater EP, Fritzemeier KH. The novel progestin drospirenone and its natural counterpart progesterone: biochemical profile and antiandrogenic potential. Contraception 1996;54:243-51.

Galliford TM, Murphy E, Williams AJ, Bassett JH, Williams GR. Effects of thyroid status on bone metabolism: a primary role for thyroid stimulating hormone or thyroid hormone? Minerva Endocrinol 2005;30:237-46.

Garner DM, Garfinkel PE. Socio-cultural factors in the development of anorexia nervosa. Psychol Med 1980;10:647-56.

Geary N. Estradiol, CCK and satiation. Peptides 2001;22:1251-63.

Geary N, Trade D, McEwen B, Smith GP. Cyclic estradiol replacement increases the satiety effects of CCK-8 in ovariectomized rats. Physiol Behav 1994;56:281-9.

Gendall KA, Bulik CM, Joyce PR, McIntosh VV, Carter FA. Menstrual cycle irregularity in bulimia nervosa. Associated factors and changes with treatment. J Psychosom Res 2000;49: 409-15.

Gibbs J, Smith GP. Satiating effect of cholecystokinin. Ann NY Acad Sci 1994;23:236-41.

Golden NH, Shenker IR. Amenorrhea in anorexia nervosa. Neuroendocrine control of hypothalamic dysfunction. Int J Eat Disord 1994;16:53-60.

Gonzalez VM, Vitousek KM. Feared food in dieting and non-dieting young women: a preliminary validation of the Food Phobia Survey. Appetite 2004;43:155-73.

Gordon CM, Nelson LM. Amenorrhea and bone health in adolescents and young women. Current Opinion in Obstetrics and Gynecology 2003;15:377-384.

Gorwood P, Kipman A, Foulon C. The human genetics of anorexia nervosa. Eur J Pharmacol 2003;480:163-70.

Grider JR. Role of cholecystokinin in the regulation of gastrointestinal motility. J Nutr 1994;124:1334S-39S.

Gupta MA, Gupta AK, Ellis CN, Voorhees JJ. Bulimia nervosa and acne may be related: a case report. Can J Psychiatry 1992;37:58-61.

Göransson K, Lidén S, Odsell L. Oral zinc in acne vulgaris: a clinical and methodological study. Acta Derm Venereol 1978;58:443-8.

Hadley SJ, Walsh BT. Gastrointestinal disturbances in anorexia nervosa and bulimia nervosa. Curr Drug Targets CNS Neurol Disord 2003;2:1-9.

Hassan MA, Killick SR. Ultrasound diagnosis of polycystic ovaries in women who have no symptoms of polycystic ovary syndrome is not associated with subfecundity or subfertility. Fertil Steril 2003;80:966-75.

Hay PJ, Bacaltchuk J. Psychotherapy for bulimia nervosa and binging. Cochrane Database Syst Rev 2001;(3): CD000562.

Herzog DB, Sacks NR. Bulimia nervosa: comparison of treatment responders vs nonresponders. Psychopharmacol Bull 1993;29:121-5.

Higley JD, Mehlman PT, Poland RE, Taub DM, Vickers J, Suomi SJ, Linnoila M. CSF testosterone and 5-HIAA correlate with different types of aggressive behaviors. Biol Psychiatry 1996;40:1067-82.

Hirschberg AL, Lindholm C, Carlström K, von Schoultz B. Reduced serum cholecystokinin response to food intake in female athletes. Metabolism 1994;43:217-22.

Hirschberg AL, Byström B, Carlström K, von Schoultz B. Reduced serum cholecystokinin and increase in body fat during oral contraception. Contraception 1996;53:109-13.

Hirschberg AL, Naessén S, Stridsberg M, Byström B, Holte J. Impaired cholecystokinin secretion and disturbed appetite regulation in women with polycystic ovary syndrome. Gynecol Endocrinol 2004;19:79-87.

Hoek HW, Bartelds AIM, Bosveld JJF, van der Graff Y, Limpens VEL, Maiwald M, Spaaij CJK. Impact of urbanization on detection rates of eating disorders. Am J Psych 1995; 152:1272-8.

Hoek HW, van Hoeken D. Rewiew of the prevalence and incidence of eating disorders. Int J Eat Disord 2003;34:383-96.

Holderness CC, Brooks-Gunn J, Warren MP. Co-morbidity of eating disorders and substance abuse: review of the literature. Int J Eat Disord 1994;16:1-34.

Howat PM, Varner LM, Hegsted M, Brewer MM, Mills GQ. The effect of bulimia upon diet, body fat, bone density and blood components. J Am Diet Ass 1989;89:929-34.

Hudson JI, Hiripi E, Pope HG Jr, Kessler RC. The prevalence and correlates of eating disorders in the National Comorbidity Survey Replication, Biol Psychiatry. 2006 Jun 30. [Epub ahead of print].

Jahanfar S, Eden JA, Nguyent TV. Bulimia nervosa and polycystic ovary syndrome. Gynecol Endocrinol 1995;9:113-7.

Joyce JM, Warren DL, Humphries LL, Smith AJ, Coon JS. Osteoporosis in women with eating disorders: comparison of physical parameters, exercise and menstrual status with SPA and DPA evaluation. J Nucl Med 1990;31:325-31.

Kaplan FS, Pertschuk M, Fallon M., Haddad, J. Osteoporosis and hip fracture in a young women with anorexia nervosa. Clin Orthop 1986;212:250-4.

Karlsson R, Lindén A, von Schoultz B. Suppression of 24-hour cholecystokinin secretion by oral contraceptives. Am J Obstet Gynecol 1992;167:58-9.

Katzman DK. Osteoporosis in anorexia nervosa: a brittle future? Curr Drug Targets CNS Neurol Dis 2003;2:11-15.

Kaye WH. Neuropeptide abnormalities. In: Halmi KA, ed. Psychobiology and treatment of anorexia nervosa and bulimia nervosa. Washington, D.C.: American Psychiatric Press, 1992.

Kaye WH, Klump KL, Frank GK, Strober M. Anorexia and bulimia nervosa. Ann Rev Med 2000;51:299-313.

Kaye WH, Guido K, Frank Carolyn C. Meltzer, and others. Altered serotonin 2A receptor activity in women who have recovered from bulimia nervosa. Am J Psych 2001;158:1152-5.

Keel PK, Mitchell JE. Outcome in bulimia nervosa. Am J Psych 1997;154:313-21.

Keel PK, Dorer DJ, Franko DL, Jackson SC, Herzog DB. Postremission predictors of relapse in women with eating disorders. Am J Psych 2005;162:2263-8.

Khan AA, Syed Z. Bone densitometry in premenopausal women: synthesis and review. J Clin Densitom 2004;7:85-92.

Khan AA, Bachrach L, Brown JP, Hanley DA, Josse RG, Kendler DL, Leib ES, Lentle BC, Leslie WD, Lewiecki EM, Miller PD, Nicholson RL, O`Brien C, Olszynski WP, Theriault MY, Watts NB. Standards and guidelines for performing central dual-energy x-ray absorptiometry in premenopausal women, men, and children. J Clin Densitom, 2004;7:51-64.

Klein DA, Boudreau GS, Devlin MJ, Walsh BT. Artificial sweetener use among individuals with eating disorders. Int J Eat Disord 2006;39:341-5.

Krattemmacher R. Drospirenone: Pharmacology and pharmacokinetics of a unique progestogen. Contraception 2000;62:29-38.

Kreipe RE, Birndorf SA. Eating disorders in adolescents and young adults. Med Clin North Am 2000;84:1027-49.

Laflamme N, Nappi RE, Drolet G, Labrie C, Rivest S. Expression and neuropeptidergic characterization of estrogen receptors (Erα and Erβ) throughout the Rat Brain: Anatomical Evidence of Distinct Roles of Each Subtype. J Neurobiol 1998;36:357-78.

Laron Z. Interactions between the thyroid hormones and the hormones of the growth hormone axis. Pediatr Endocrinol Rev 2003;2:244-9.

Lee M-C and Lee S-H. Premenstrual tension syndrome with periodic bulimia nervosa: report of a case and review of the litterature. J Formosan Med Assoc 1992;7:716-20.

Legroux-Gerot I, Vignau J, Collier F, Cortet B. Bone loss associated with anorexia nervosa. Joint Bone Spine 2005;72:489-95.

Leombruni P, Amianto F, Delsedime N, Gramaglia C, Abbate-Daga G, Fassino S. Citalopram versus fluoxetine for the treatment of patients with bulimia nervosa: a single-blind randomized controlled trial. Adv Ther 2006;23:481-94.

Lerner UH. Bone remodelling in post-menopausal osteoporosis. J Dent Res 2006;85:584-95.

Levy AB, Malarkey WB. Growth hormone and somatomedin-C in bulimia. Psychoneuroendocrinology 1988;13:359-62.

Levy AB, Neuroendocrine profile in bulimia nervosa. Biol Psychiatry 1989;25:98-109.

Liang YQ, Akishita M, Kim S, Ako J, Hashimoto M, Iijima K, Ohike Y, Watanabe T, Sudoh N, Toba K, Yoshizumi M, Ouchi Y. Estrogen receptor beta is involved in the anorectic action of estrogen. Int J Obes Rel Metab Disord 2002;26:1103-9.

Lilenfeld LR, Kaye WH, Greeno CG, Merikangas KR, Plotnicov K, Pollice C, Rao R, Strober M, Bulik CM, Nagy L. A controlled family study of anorexia nervosa and bulimia nervosa: psychiatric disorders in first-degree relatives and effects of proband comorbidity. Arch Gen Psychiatry 1998;55:603-10.

Lilenfeld LR, Stein D, Bulik CM, Strober M, Plotnicov K, Pollice C, Rao R, Merikangas KR, Nagy L, Kaye WH. Personality traits among currently eating disordered, recovered and never ill first-degree female relatives of bulimic and control women. Psychol Med 2000;30:1399-410.

Lilenfeld LR, Wonderlich S, Riso LP, Crosby R, Mitchell J.Eating disorders and personality: a methodological and empirical review 2006;26:299-320. Lindén A. Role of cholecystokinin in feeding and lactation. Acta Physiol Scand 1989;137:1-49.

Lindén A, Uvnäs-Moberg K, Forsberg G, Bednar I, Södersten P. Involvement of cholecystokinin in food intake: III, Oestradiol potentiates the inhibitory effect of cholecystokinin octapeptide on food intake in ovariectomized rats. J Neuroendocrinol 1990;2:797-801.

Lindén A, Södersten P. Relationship between the concentration of cholecystokinin-like immunoreactivity in plasma and food intake in male rats. Physiol Behav 1990;48:859-63.

Marchal-Victorion S, Vionnet N, Escrieut C, Dematos F, Dina C, Dufresne M, Vaysse N, Pradayrol L, Froguel P, Fourmy D. Genetic, pharmacological and functional analysis of cholecystokinin-1 and cholecystokinin-2 receptor polymorphism in type 2 diabetes and obese patients. Pharmacogenetics 2002;12:23-30.

McCluskey SE, Evans C, Lacey JH, Pearce JM, Jacobs, H. Polycystic ovary syndrome and bulimia. Fertil Steril 1991;55:287-91.

McCluskey SE, Lacey JH, Pearce JM. Binge-eating and polycystic ovaries. Lancet 1992; 340:723.

McIlwain HH. Glucocorticoid-induced osteoporosis: pathogenesis, diagnosis, and management. Prev Med 2003;36:243-9.

McSherry J. Bulimia nervosa and acne. Can J Psychiatry 1992;37:731-2.

Michelmore K, Ong K, Mason S, Bennett S, Perry L, Vessey M, Balen A, Dunger D. Clinical features in women with polycystic ovaries: relationships to insulin sensitivity, insulin gene VNTR and birth weight. Clin Endocrinol 2001;55:439-46.

Migliaccio S, Newbold RR, Bullock BC, Jefferson WJ, Sutton FG, McLachlan JA, Korach KS. Alterations of maternal estrogen levels during gestation affect the skeleton of female offspring. Endocrinology 1996;137:2118-25.

Miller KK, Lee EE, Lawson EA, Misra M, Minihan J, Grinspoon SK, Gleysteen S, Mickley D, Herzog D, Klibanski A. Determinants of skeletal loss and recovery in anorexia nervosa. J Clin Endocrinol Metab 2006;91:2931-7.

Milsom I, Lete I, Bjertnaes A, Rokstad K, Lindh I, Gruber CJ, Birkhauser MH, Aubeny E, Knudsen T, Bastianelli C. Effects on cycle control and bodyweight of the combined contraceptive ring, NuvaRing, versus an oral contraceptive containing 30 microg ethinyl estradiol and 3 mg drospirenone. Hum Reprod 2006;21:2304-11.

Misra M, Klibanski A. Anorexia nervosa and osteoporosis. Rev Endocr Metab Disord 2006 Sep14;[Epub ahead of print].

Mitchell JE, Crow S. Medical complications of anorexia and bulimia nervosa. Curr Opin Psych 2006;19:438-43.

Moran L, Norman RJ. Understanding and managing disturbances in insulin metabolism and body weight in women with polycystic ovary syndrome. Best Pract Res Clin Obstet Gynaecol 2004;18:719-36.

Morgan JF, McCluskey SE, Brunton JN, Lacey JH. Polycystic ovarian morphology and bulimia nervosa: a 9-year follow-up study. Fertil Steril 2002;77:928-31.

Mortola JF, Girton L, Yen SS. Depressive episodes in premenstrual syndrome. Am J Obstet Gynecol 1989;161:1682-7.

Mortola JF, Rasmussen DD, Yen SS. Alterations of the adrenocorticotropin-cortisol axis in normal weight bulimic women: evidence for a central mechanism. J Clin Endocrinol Metab 1989;68:517-22.

Monteleone P, Luisi M, Colurcio B, Casarosa E, Monteleone P, Ioime R, Genazzani AR, Maj M. Plasma levels of neuroactive steroids are increased in untreated women with anorexia nervosa or bulimia nervosa Psychosom Med 2001; 63:62-8.

Monteleone P, Martiadis V, Colurcio B, Maj M. Leptin secretion is related to chronicity and severity of the illness in bulimia nervosa. Psychosom Med 2002;64:874-9.

Monteleone P, Martiadis V, Fabrazzo M, Serritella C, Maj M. Ghrelin and leptin responses to food ingestion in bulimia nervosa: implications for binge-eating and compensatory behaviours. Psychol Med 2003;33:1387-94.

Monteleone P, Martiadis V, Rigamonti AE, Fabrazzo M, Giordani C, Muller EE, Maj M. Investigation of peptide YY and ghrelin responses to a test meal in bulimia nervosa. Biol Psychiatry 2005;57:926-31.

Muhn P, Fuhrmann U, Fritzemeier KH, Krattenmacher R, Schillinger E. Drospirenone: a novel progestogen with antimineralocorticoid and antiandrogenic activity. Ann N Y Acad Sci 1995;761:311-35.

Mury M, Verdoux H, Bourgeois M. Comorbidity of bipolar and eating disorders. Epidemiologic and therapeutic aspects. Encephale 1995;21:545-53.

Münster K, Helm P, Schmidt L. Secondary amenorrhoea: prevalence and medical contact-a cross-sectional study from a Danish county. Br J Obstet Gynecol 1992;99:430-3.

Nakazato M, Hashimoto K, Shiina A, Koizumi H, Mitsumoti M, Imai M, Shimizu E, Iyo M. No changes in serum ghrelin levels in female patients with bulimia nervosa. Prog Neuro-psychopharmacol Biol Psychiatry 2004;28:1181-4.

Neudeck P, Jacoby GE, Florin I. Dexamethasone suppression test using saliva cortsol measurement in bulimia nervosa. Physiol Behav 2001;72:93-8.

Neumark-Sztainer D, Story M, French SA. Covariations of unhealthy weight loss behaviors and other high-risk behaviors among adolescents. Arch Pediatr Adolesc Med 1996;150:304-8.

Newman MM, Halmi KA. Relationship of bone density to estradiol and cortisol in anorexia nervosa and bulimia. Psychiatry Res 1989;29:105-12.

Newton JR, Freeman CP, Hannan WJ, Cowen S. Osteoporosis and normal weight bulimia nervosa – which patients are at risk? J Psychosom Res 1993;3:239-47.

Nielsen S. Epidemiology and mortality of eating disorders. Psychiatr Clin North Am 2001;24:201-14.

Niiyama S, Happle R, Hoffmann R. Influence of estrogens on the androgen metabolism in different subunits of human hair follicles. Eur J Dermatol 2001;11:195-8.

Nilsson LO, Boman A, Sävendahl L, Grigelioniene G, Ohlsson C, Ritzen EM, Wroblewski JJ. Demonstration of estrogen receptor-β immunoreactivity in human growth plate cartilage. J Clin Endocrinol Metab 1999;84:370-3.

Nuti R, Martini G, Righi G, Frediani B & Turchetti V. Comparison of total-body measurements by dual-energy X-ray absorptiometry and dual-photon absorptiometry. J Bone Min Res 1991;6:681-7.

O'Brien KM, Vincent NK. Psychiatric comorbidity in anorexia and bulimia nervosa: nature, prevalence, and causal relationships. Clin Psychol Rev 2003;23:57-74.

Oelkers W. Drospirenone, a progestogen with antimineralocorticoid properties: a short review. Mol Cell Endocrinol 2004;217:255-61.

Ogawa S, Hosoi T, Shiraki M, Orimo H, Emi M, Muramatsu M, Ouchi Y, Inoue S. (a). Association of estrogen receptor beta gene polymorphism with bone mineral density. Biochem Biophys Res Commun 2000;269:537-41(a).

Ogawa S, Emi M, Shiraki M, Hosoi T, Ouchi Y, Inoue S. (b). Association of estrogen receptor beta (ESR2) gene polymorphism with blood pressure. J Hum Genet 2000; 45:327-30.

Olmsted MP, Kaplan AS, Rockert W. Defining remission and relapse in bulimia nervosa. Int J Eat Disord 2005;38:1-6.

Olszewski PK, Grace MK, Billigton CJ, Levine AS. Hypothalamic paraventricular injections of ghrelin: effects on feeding and c-Fos immunoreactivity. Peptides 2003;919-23.

Osculati A, Castiglioni C. Fatal liver complications with flutamide. Lancet 2006;367:1140-1.

Pekhlivanov B, Mitkov M, Ivancheva Kh., Amaliev I. Use of yasmin in the treatment of women with polycystic ovary syndrome. Akush Ginekol 2006;45:25-9.

Raphael FJ, Rodin DA, Peattie A, Bano G, Kent A, Nussey SS, Lacey JH. Ovarian morphology and insulin sensitivity in women with bulimia nervosa. Clin Endocrinol 1995;43:451-5.

Recker RR, Davies KM, Hinders SM, Heaney RP, Stegman MR, Kimmel DB. Bone gain in young adult women. JAMA 1992;268:2403-8.

Resch M, Szendei G, Haasz PJ. Bulimia from a gynecological view: hormonal changes.Obstet Gynaecol 2004;24: 907-10.

Rigotti NA, Neer RM, Skates SJ, Herzog DB, Nussbaum SR. The clinical course of osteoporosis in anorexia nervosa. A longitudinal study of cortical bone mass. JAMA 1991;265:1133-8.

Rivera-Gallardo MT, Ma del Socorro PC, Barriguete-Melendez JA. Eating disorders as risk factors for osteoporosis. Salud Publica Mex 2005;47:308-18.

Rodriguez-Cano T, Beato-Fernandez L, Belmonte-Llario A. New contributions to the prevalence of eating disorders in Spanish adolescents: detection of false negatives. Eur Psychiatry 2005;20:173-8.

Rodriguez Martin A, Novalbos Ruiz JP, Martinez Nieto JM, Escobar Jimenez L, Castro De Haro AL. Epidemiological study of the influence of family and socioeconomic status in disorders of eating behavior. Eur J Clin Nutr 2004;58:846-52.

Rosen CJ, Bilezikian JP. Anabolictheraphy for osteoporosis. J Clin Endocrinol Metab 2001;86:957-64.

Rosenkranz K, Hinney A, Ziegler A, Hermann H, Fichter M, Mayer H, Siegfried W, Young JK, Remschmidt H, Hebebrand J. Systematic mutation screening of the estrogen receptor beta gene in probands of different weight extremes: identification of several genetic variants. J Clin Endocrinol Metab 1998;83:4524-7.

The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2004 Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril 2004;81:19-25.

Russel GFM: Bulimia nervosa: an ominous variant of anorexia nervosa. Psychol Med 1979; 9:429-48.

Russell JD. Update in eating disorders. Aust N Z J Med 1996;26:819-23.

Russell J, Hooper M, Hunt G. Insulin response in bulimia nervosa as a marker of nutritional depletion. Int J Eat Disord 1996;20:307-13.

Sansone RA, Levitt JL, Sansone LA. The prevalence of personality disorders among those with eating disorders. Eat Disord 2005;13:7-21.

Santonastaso P, Mondini S, Favaro A. Are fashion models a group at risk for eating disorders and substance abuse? Psychother Psychosom 2002;71:168-72

Schweiger U, Pirke KM, Laessle RG, Fichter MM. Gonadotropin secretion in bulimia nervosa. J Clin Endocrinol Metab 1992;74:1122-7.

Shenker J. Stress and infertility. Akush Ginekol 1993:39-42.

Sica DA. Drospirenone: an antihypertensive in waiting. Hypertension 2006;48:205-6.

Silberg JL, Bulik CM. The developmental association between eating disorders symptoms and symptoms of depression and anxiety in juvenile twin girls. J Child Psychol Psychiatry. 2005; 46:1317-26.

Sitruk-Ware R. Pharmacology of different progestogens: the special case of drospirenone. Climacteric 2005;8:4-12.

Steinhausen, HC. Eating disorders. In: Steinhausen, HC, Verhulst FC, Eds. Risks and outcomes in developmental psychopathology. Oxford, England: Oxford University Press 1999, pp 210-30.

Sundblad C, Bergman L, Eriksson E. High levels of free testosterone in women with bulimia nervosa. Acta Psychiatr Scand 1994;90:397-8.

Sundarrajan C, Liao W, Roy AC, Ng, SC. Association between estrogen receptor-β gene polymorphisms and ovulatory dysfunctions in patients with Mentrual Disorders. J Clin Endocrinol Metab 2001;86:135-39.

Sundblad C, Landen M, Eriksson T, Bergman L, Eriksson E. Effects of the androgen antagonist flutamide and the serotonin reuptake inhibitor citalopram in bulimia nervosa: a placebo-controlled pilot study. J Clin Psychopharmacol 2005;25:85-8.

Sundgot-Borgen J, Bahr R, Falch JA, Schneider LS. Normal bone mass in bulimic women. J Clin Endocrinol Metab 1998;83:3144-9.

Sundgot-Borgen J, Torstveit MK.Prevalence of eating disorders in elite athletes is higher than in the general population. Clin J Sport Med 2004;14:25-32.

Syed F, Khosla S. Mechanisms of sex steroid effects on bone. Biochem Biophys Res Commun 2005;328:688-96.

Södergård R, Bäckström T, Shanbag V, Carstensen H. Calculation of free and bound fractions of testosterone and estradiol-17ß to plasma proteins at body temperature. J Steroid Biochem 1982;18:801-4.

Tanaka M, Naruo T, Muranaga T, Yasuhara D, Shiiya T, Nakazato M, Matsukura S, Nozoe S. Increased fasting plasma ghrelin levels in patients with bulimia nervosa. Eur J Endocrinol 2002;146:R1-3.

Treasure JL, Fogelman I, Russel GF. Osteopaenia of the lumbar spine and femoral neck in anorexia nervosa. Scott Med J 1986;31:206-7.

Treasure JL, Wheeler M, King EA, Gordon PAL, Russell GFM. Weight gain and reproductive functions: ultrasonographic and endocrine features in anorexia nervosa. Clin Endocrinol 1988;29:607-16.

Tulchinsky D, Chopra IJ. Estrogen-androgen imbalance in patients with hirsutism and amenorrhea. J Clin Endocrinol Metab 1994;39:164-9.

Vandereycken W, Hoek HW. Are eating disorders culture-bound syndromes? In Halmi KA Eds. Psychobiology and Treatment of Anorexia Nervosa and Bulimia Nervosa. Washington DC: American Psychopathological Association 1993, pp 19-36.

Vaz FJ. Outcome of bulimia nervosa: prognostic indicators. J Psychosom Res 1998;45:391-400.

Vitousek KM, Stumpf RE. Difficulties in the assessment of personality traits and disorders in eating-disordered individuals. Eat Disord 2005;13:37-60.

Walsh BT, Hadigan CM, Kissileff HR., LaChaussee JL. Bulimia nervosa: a syndrome of feast and famine. In: Andersson GH, Kennedy SH Eds. The biology of feast and famine. San Diego: Academic Press 1992, pp 3-20.

Westberg L, Baghaei F, Rosmond R, Hellstrand M, Landén M, Jansson M, Holm G, Björntorp P, Eriksson E. Polymorphisms of the androgen receptor gene and the estrogen receptor β gene are associated with androgen levels in women. J Clin Endocrinol Metabol 2001;86:2562-8.

White WB, Pitt B, Preston RA, Hanes V. Antihypertensive effects of drospirenone with 17beta-estradiol, a novel hormone treatment in postmenopausal women with stage 1 hypertension. Circulation 2005;112:1979-84.

Woodside DB, Garfinkel PE, Lin E, Goering P, Kaplan AS, Goldbloom DS, Kennedy SH. Comparisons of men with full or partial eating disorders, men without eating disorders, and women with eating disorders in the community. Am J Psych 2001;158:570-4.

Wren AM, Seal LJ, Cohen MA, Brynes AE, Frost GS,Murphy KJ, Dhillo WS, Ghatei MA, Bloom SR. Ghrelin enchances appetite and increases food intake in humans. J Clin Endocrinol Metab 2001;86:5992-5.

Zipfer S, Seibel MJ, Löwe B, Beumont PJ, Kasperk C & Herzog W. Osteoporosis in eating disorders: A follow-up study of patients with anorexia nervosa and bulimia nervosa. J Clin Endocrinol Metab 2001;86:5227-33.

Östlund H, Keller E, Hurd YL. Estrogen receptor gene expression in relation to neuropsychiatric disorders. Ann N Y Acad Sci 2003;1007:54-63.

PAPERS I-IV