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REPRODUCTIVE HEALTH IN ADOLESCENT GIRLS WITH SPECIAL EMPHASIS ON MENSTRUAL DISORDERS, BONE HEALTH, SEXUALITY AND SOCIAL FACTORS

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Will you listen to me
Dare I tell you
Can I trust you not to betray me

Who cares?
Marianne Volckerts 2006 (translated from Swedish)

To Erik, beloved son, and others who with love, care and humor make our lives happy

Reproductive health in adolescent girls with special emphasis on menstrual disorders, bone health, sexuality and social factors

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Abstract

Menstrual disturbances are common during adolescence, especially within the first 1-3 years after menarche, and are often explained by immaturity of the hypothalamic-pituitary-gonadal axis. However, little is known about the underlying mechanisms, long-term medical consequences and other reproductive health issues in teenagers.

The general aim of this thesis was to investigate aspects of reproductive health in adolescent girls and boys visiting a youth clinic. Endocrine mechanisms of menstrual disorders, eating behavior and long-term medical consequences, including bone mass, were specifically evaluated. The aim was also to study sexuality and social factors in adolescent girls in comparison with boys.

The mechanisms of menstrual disorders were studied in 203 girls, 117 with secondary amenorrhea and 86 with oligomenorrhea. Hypothalamic amenorrhea dominated in the girls with secondary amenorrhea, whereas hyperandrogenism, i.e. polycystic ovary syndrome (PCOS) was the main mechanism in the oligomenorrheic group. Eating disorders were common (53%); anorectic behavior in the secondary amenorheic group and bulimic type more frequent in the oligomenorrheic group. A follow-up study was performed six years later among 87 of the originally investigated girls; 52 with previous secondary amenorrhea and 35 with previous oligomenorrhea. Menstrual disturbances were still present in 62% of the subjects not using hormonal contraception; 59% of them fulfilled the criteria for PCOS, which was the main mechanism explaining persistent menstrual disorder. Recovery from anorectic eating beahvior was a strong predictor of resumption of regular menstruation.

Bone mineral density (BMD) was assessed in the 87 women participating in the follow-up and measured with whole-body dual energy X-ray absorptiometry (DXA). The frequency of osteopenia/osteoporosis was 52%; 3 women had osteoporosis. Those with previous secondary amenorrhea had significantly lower BMD in pelvis and lumbar spine than those with previous oligomenorrhea. Restrictive eating disorder in adolescence was the strongest predictor of low BMD and the most important counteraction was high physical activity at follow-up and a BMI $\geq$ 22. Persistent menstrual dysfunction (PCOS) was associated with a lower frequency of osteopenia. Aspects of life quality and sexuality were studied in 480 girls and 108 boys by means of a questionnaire survey. Girls were less satisfied with life, their bodies and sexuality than boys. Weight control behavior was notably common among girls and may indicate an eating disorder. The overall results show that a proportion of the girls and boys constitute a risk group for adverse reproductive health due to alcohol abuse, non-use of contraception and family problems.

We conclude that eating disorder is the most important underlying cause of menstrual dysfunction in adolescence. PCOS was the main mechanism explaining persistent menstrual disorder in adulthood. Anorectic behavior in adolescence was the strongest predictor of low bone mass at follow-up. We found clear gender differences in life quality, life style and sexual experiences. A youth clinic with interdisciplinary competence provides good opportunity for adequate medical care and treatment of reproductive health issues in girls and boys.

Key words: adolescence, menstrual disorders, PCOS, eating disorders, bone mineral density, physical activity, sexuality, gender differences, family relations, life quality, life style.

LIST OF PUBLICATIONS

This thesis is based on the following papers, which will be referred to in the text by the Adolescent study (I), Follow-up study (II), Bone mineral density at follow-up (III), Life quality and sexuality (IV) or just by their Roman numerals.

I. Marianne Wiksten-Almströmer, Angelica Lindén Hirschberg, Kerstin Hagenfeldt. *Menstrual disorders and associated factors among adolescent girls visiting a youth clinic.* Acta Obstet Gynecol Scand 2007;86(1):65-72.

II. Marianne Wiksten-Almströmer, Angelica Lindén Hirschberg, Kerstin Hagenfeldt. *Prospective follow-up of menstrual disorders in adolescence and prognostic factors.* Acta Obstet Gynecol Scand 2008;87(11):1162-8.

III. Marianne Wiksten-Almströmer, Angelica Lindén Hirschberg, Kerstin Hagenfeldt. *Reduced bone mineral density in adult women diagnosed with menstrual disorders during adolescence.* Accepted for publication in Acta Obstet Gynecol Scand 2009-02-23.

IV. Marianne Wiksten-Almströmer. *Gender differences in sexuality and life quality among young people visiting a Swedish youth clinic.* Int J Adolesc Med Health, 2009;21(1):29-39.

CONTENTS

LIST OF ABBREVIATIONS

17-OHP	17-hydroxyprogesterone
ACTH	adrenocorticotropic hormone
APA	American Psychiatric Association
ASRM	American Society for Reproductive Medicine
BMC	bone mineral content
BMD	bone mineral density
BMI	body mass index
CI	confidence interval
CRH	corticotropin-releasing hormone
DHEAS	dehydroepiandrosterone sulfate
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, fourth edition
DXA	dual-energy X-ray absorptiometry
EDI	Eating disorder inventory test
EDNOS	eating disorders not otherwise specified
ESHRE	European Society of Human Reproduction and Embryology
FSUM	Föreningen för Sveriges Ungdomsmottagningar (Association of Sweden's Youth Centres)
FIA	fluoroimmunoassay
FSH	follicle-stimulating hormone
GABA	gamma amino butyric acid
GH	growth hormone
GnRH	gonadotropin releasing hormone
HPA-axis	hypothalamic-pituitary-adrenal axis
HPG-axis	hypothalamic-pituitary-gonadal axis
IGF-I	insulin like growth factor I
LBM	lean body mass
LH	lutenizing hormone
MRI	Magnetic Resonance Imaging
OCs	combined hormonal oral contraceptives
OM	oligomenorrhea
OR	odds ratio
PCOS	polycystic ovary syndrome
PCO	polycystic ovaries
RIA	radioimmunoassay
SA	secondary amenorrhea
SBU	Statens Beredning för Medicinsk Utvärdering (The Swedish Council on Technology Assessment in Health Care)
SD	standard deviation
SHBG	sex hormone-binding globulin
TSH	thyroid-stimulating hormone
T4	thyroxine
WHO	World Health Organization

1 INTRODUCTION

Adolescence is a dynamic period in life. A negative outcome has serious potential consequences for future health. Conversely, adolescence can be a good period for promoting a healthy lifestyle. The general circumstances for teenagers vary over time, due to changes in the family, school and society. The National Board of Health and Welfare currently evaluates young people's health in Sweden on a regular basis. When an adolescent seeks help for a health problem, the ability to evaluate and to provide appropriate treatment will depend on the type of clinic and its resources. At a youth clinic, the staff has particular competence to take good care of adolescents.

1.1 YOUTH CLINICS

The setting of this thesis is a youth clinic in Stockholm. Ever since the first unit opened in 1970, youth clinics in Sweden have deliberately integrated physical, mental and social factors in their working methods (FSUM 2002). Contraception, unwanted pregnancies, sexually transmitted infections (STI), body development and sexual relations are common reasons for visits. Youth clinics play an important role in interventions aimed at improving the mental and social health of young people, offering cognitive counseling as well as support in the event of family and relationship problems. There are currently about 200 youth clinics in Sweden, visited by more than 250,000 young people each year.

Eighty nine per cent of the visitors are girls (Socialstyrelsen 2005), even though there are no alternative health care facilities for young men. One explanation for the predominance of girls could be that the youth clinics provide hormonal contraceptives. Another hypothesis is that girls may be more inclined to seek help or have different needs for consultation.

1.2 ADOLESCENCE

1.2.1 Development

Adolescence is the period from onset of puberty until adulthood. The period is characterized by intense physical, psychological, sexual and social development.

1.2.1.1 Physical

Adolescence is a period of rapidly increasing height. The growth will accelerate up to an average of 8 cm per year for girls and 10 cm for boys (Tanner 1962). In girls, the peak velocity will be about one year before menarche (Speroff et al. 2005 a). At menarche, most girls have reached 90% of their predicted final height. The age of menarche for Swedish girls is 12.8 years (Lindgren et al. 1991). Boys reach their target height about two years later than girls (Speroff et al. 2005 a). Body weight, height and body mass index (BMI, kg/m²) all increase during the growth period. In mid adolescence, the amount of fat increases in girls and its distribution changes towards a female type. Among boys there is a more obvious increase in muscle mass (Tanner et

al. 1981, Loomba-Albrecht & Styne 2009). The changes in body composition continue to develop in late adolescence. Other obvious bodily changes are the emergence of pubic hair and breast development, which start in early adolescence. In boys there is also enlargement of the testicles and penis.

1.2.1.2 Psychological

Psychological development from child to adult can be divided into three stages: early adolescence 10-13 years, mid adolescence 14-17 years and late adolescence 18-20 years (Piaget 1966). Boys develop one to two years later than girls. A transition in cognitive development from concrete to abstract thinking in early adolescence enables the individual to use hypothetical reasoning. Adolescents have a tendency to place themselves in the centre of things, surrounded by an "imaginary audience", focusing attention on themselves. The onset of abstract thinking is also conducive to egocentrism or self-centred thinking, including the belief that natural laws do not apply to oneself ("personal fable"). Omnipotence, a feeling of being invulnerable, together with a lively imagination and opposition to the family are characteristic of early adolescence. Experimentation and risk-taking behavior can therefore be seen as a part of normal development (Berg-Kelly 1998). In mid adolescence, further emotional and cognitive development lead to the formation of an identity. During this period, the teenager separates from the influence of authorities, parents, teachers and health workers. In late adolescence, this process of separation-individuation leads to a more stable identity (Blos 1979). The young person is more apt to listen to adults and becomes increasingly aware of the difficulties of living an adult life. A gender difference is discernible in the reaction to problems. Girls react more inwardly, with symptoms such as depression, whereas boys are more likely to act out in their surroundings (Ostrov et al.1989).

1.2.1.3 Sexual

Adolescence is a period with an increasing focus on sexuality, own sexual reactions, as well as sexual relations with partners, of the other or the same gender. This focus may meet developmental needs such as autonomy, affiliation and identity (Irwin 1990). In Sweden, teenage sexuality is generally accepted. Free contraceptives are readily available and abortion is a free decision up to the 18th week of pregnancy. A review of several Swedish studies reports that the mean age of first coitus has hardly changed in the past decade: around 16 for both girls and boys (Forsberg 2005). However, the age of first coitus is dependent on social factors, as described in the study by Edgardh and co-worker, indicating that girls and boys attending a vocational school program have earlier first coitus than those attending an educational program (Edgardh et al. 1999). Experimentation and risk-taking are a part of adolescent behavior. The advantages of protection from unwanted consequences of intercourse, i.e. pregnancy or STI, may conflict with this. That is even more the case in early adolescence. Girls with first coitus early in life are reported to have a more hazardous sexual behavior, which includes more partners and less use of contraception and STI-protection (Andersson-Ellström 1996)

1.2.1.4 Social

Young people make a social journey from being a part of a family to becoming an independent person with ability to live and support themselves. Most young people in Sweden attend the nine-year compulsory school, followed by upper-secondary school or high school up to the age of 19. Adolescence is also a period when experiences with the family are put on trial and teenagers try to find out who they are and how they want to live their lives. This involves experimenting with different life styles, some of which will be negative for health (Berg-Kelly 1995). The term "risk-taking behavior" is used when large risks are taken repeatedly, and includes a variety of behaviors, such as drug abuse, truancy and shop-lifting. Adolescents engaged in risk-taking behaviors may also be at risk of reproductive health problems, due to careless sexual behavior (Andersson-Ellström 1998). Support from the family is crucial for avoiding a risky lifestyle. Interventions by the community have also been reported to have an effect on health behavior (Berg-Kelly et al.1997).

1.2.1.5 Approach to problems

Teenagers may have difficulties in realizing and describing their problems. When an adolescent has a problem, her/his maturity must be taken into account when explaining the causes and consequences and how the young person could be involved in the treatment. Maturity differs between individuals of the same age but for practical purposes an overview of different characteristics and age-specific approaches to health problems has been suggested, as shown in Table 1.

Table 1. Psychosocial development during adolescence and age-specific approaches to health problems.

	Psychosocial development			Age-specific approach to a problem
Early adolescence: Girls 10-13 years Boys 11-14 years	Abstract thinking begins. Rich fantasy life (imaginary audience, personal fable).	Pubertal development ends. Increased awareness of the body.	Psychological separation from the family begins.	Discuss the normalities and give the young person a very limited responsibility for the problem. Be very concrete. Support from the family is important. Assist in conflicts within the family.
Mid adolescence: Girls 13-16 years Boys 14-17 years	Cognitive thinking develops even more.	Growing interest in a possible partner.	Real separation from the family.	Discuss the problem. Inform about sexual aspects of the problem. The young person may be given more responsibility for the treatment of the problem. Peers and friends could be a help.
Late adolescence: Girls 16-21 years Boys 17-21 years	More established personality. Able to again seek advice from authorities.	Realistic plans for future occupation.	A close relation to someone outside the family can be developed.	Realistic discussion about the problem's consequences for future health. Now able to choose a lifestyle to achieve certain goals.

Adopted from Berg-Kelly 1995

1.2.2 Hormonal development in adolescent girls

In prepubertal girls, the first distinct hormonal change is the increased production of adrenal androgens. Pubertal development is controlled by a gradual maturation of the hypothalamic-pituitary-gonadal (HPG) system. An increased secretion of hypothalamic gonadotropin releasing hormone (GnRH) will be followed by increased pituitary secretions of lutinizing hormone (LH) and follicle-stimulating hormone (FSH). The first LH pulses are seen during sleep and gradually extend through the day. The rise in FSH and LH levels is followed by an increase in circulating estradiol produced in the ovaries. When estrogen increases, breast development, female fat distribution and maturation of the genital tract will occur. The increase in androgens from the ovary and from the adrenal cortex will cause pubic and axillary hair growth, oily skin and acne. Growth hormone (GH) and gonadal estrogen are vital for the pubertal growth spurt. The accelerated growth is also associated with an increase in circulating levels of the anabolic hormone insulin-like growth factor-I (IGF-I). Estrogen is later responsible for the closure of the long bones that occurs at the end of the growth period (Speroff et al 2005 a).

The timing of puberty is mainly genetically determined. Delayed puberty, including delayed bone age and menarche, is associated with specific diseases and other multiple factors. Factors that have been considered are high intensity of physical activity, low amount of body fat, psychological stress and inadequate diet (Warren 1980, Frish et al. 1981, Mansfiled & Emans 1989, Lindholm et al. 1994). A prolonged hypothalamic inhibition of the GnRH secretion has been suggested as the fundamental factor behind delayed puberty (Theintz et al. 1993). Frish (1985) suggested that a critical percentage of body fat (17%) is required to achieve menarche, but this specific figure has been questioned.

1.3 MENSTRUAL DISTURBANCES

Menstrual disturbances are common among adolescents (Slap 2003). The definition of primary amenorrhea is no period by age 16 or later. In a woman who has been menstruating, the definition of secondary amenorrhea is the absence of periods for at least three previous cycles, or 6 months of amenorrhea (Speroff et al. 2005 b). Oligomenorrhea is defined as irregular menstruation intervals of 6 to 24 weeks with a maximum of 4 menstruations during a 6-month period. The prevalence of secondary amenorrhea in young girls has been reported to 2.6 - 8.5% (Pettersson et al. 1973, Bachmann et al. 1982, Munster et al. 1992) and irregular menstrual periods to 11.3 - 26.7% (Bachmann et al. 1982, Demir et al. 2000). Menstrual irregularities in teenagers during the first years after menarche are often explained by immaturity of the HPG-axis and are considered to normalize with increasing stability of the axis (Apter et al. 1978, Mansfield & Emans 1984). However, information about the causes of menstrual disturbances later during adolescence is scarce. Most of the studies are cross-sectional surveys (Bachmann & Kemmann 1982, van Hooff et al. 1998) and only a few have investigated underlying endocrine mechanisms of menstrual disturbances in young girls (Reindollar et al. 1981, Reindollar et al. 1986). The mechanisms of primary amenorrhea could be the same as for secondary amenorrhea but chromosomal aberrations and congenital malformations could also be involved (Speroff et al. 2005b). Lack of menstruation or irregular periods prompt help-seeking by the girl or her parents. School health personnel also ask about menstrual periods and irregularity will often lead to referral for evaluation.

1.4 HYPOTHALAMIC AMENORRHEA

1.4.1 Mechanism

Hypothalamic inhibition of the reproductive system, i.e. hypothalamic amenorrhea, is considered to be a common mechanism for menstrual disorders in young women (Golden & Carlson 2008). This disorder is characterized by low levels of gonadotropins, particularly of LH, resulting in low estradiol levels (Figure 1). The most common underlying causes of hypothalamic amenorrhea are weight loss, eating disorders, strenuous exercise and stress (Golden & Carlson 2008).

Several hormones and mechanisms are involved in the HPG-axis; for a summary, see Figure 1. GnRH, produced by neurons in hypothalamus, stimulates the synthesis and secretion of LH and FSH in the pituitary. The GnRH is released in pulses in response to serum levels of steroids. Secretion of GnRH is also regulated by a number of

neurotransmitters, including dopamine, endogenous opioids, norepinephrine, gamma amino butyric acid (GABA), and corticotropin-releasing hormone (CRH). The modulation of GnRH release is complex, with interactions between a number of regulatory systems. The hypothalamus is sensitive to circulating levels of estrogen, which inhibit GnRH secretion by the hypothalamus (the "negative feedback loop"). The negative feedback loop is present in the fetus and is active throughout childhood and adolescence. The "positive feedback loop", in which a critical level of estrogen stimulates pulses of GnRH, which triggers the LH-surge and ovulation, does not develop until later in puberty. Leptin, a protein produced by the adipocytes, acts on the hypothalamus to regulate food intake, energy expenditure, and body weight. Leptin is also suggested to influence GnRH secretion (Thong & Graham 1999, Miller et al 2004).

The most important cause of inhibition of the HPG-axis is low energy availability, which leads to a disruption of GnRH pulsatility (Loucks 2003). This will result in reduced LH and FSH secretion, resulting in low production of sex steroid from the ovaries, which will cause anovulation and oligo/amenorrhea (Figure 1) (Goodman & Warren 2005, Redman & Loucks 2005). Low available energy and stress will activate the hypothalamic-pituitary-adrenal (HPA) axis, thereby resulting in an increased secretion of CRH and cortisol, which inhibit the reproductive system (Lindholm et al. 1995, Laughlin & Yen 1996, Miller et al. 2004). CRH is responsible for activation of central endogenous opioid activity, in particular that of beta-endorphin. Both CRH and endogenous opioids directly inhibit GnRH release by the hypothalamus (Barbarino et al. 1989). A hypometabolic state is reflected by high levels of IGF-binding protein (IGFBP-1), low levels of insulin and IGF-I, which in turn may decrease LH secretion (Laughlin & Yen 1996). Leptin may be the link between low fat mass and menstrual disorders. The current notion is that in both anorexia nervosa and exercised-induced amenorrhea, amenorrhea is an adaptive response to energy deficit, mediated in part by leptin (Misra et al. 2004, Haas et al. 2005).

1.4.2 Weight loss, exercise and inadequate nutrition

Weight loss and strenuous exercise have been reported to be important etiological factors behind menstrual disturbances in college students. Energy deficiency is associated with hypothalamic inhibition of the reproductive axis, resulting in inadequate luteal phases and oligo/amenorrhea. Energy requirements are directly proportional to body size and degree of physical activity. Reference values for energy intake in women according to the Swedish Nutrition Recommendations (SNR) 1997 are given in Table 2. According to these recommendations, the energy intake should consist of 55-60% carbohydrates, 30% protein and 10-15% fat. A diet rich in fibers and low in fat and proteins is associated with decreased levels of sex hormones (Adlercreutz 1991), due to the influence of the enterohepatic circulation and the content of phytoestrogens competing with the estrogen receptors.

Table 2. Reference values in kilocalories (kcal) for females according to Swedish Nutrition Recommendations 1997.

age	mean	range
11 – 14 years	2020 kcal	1180 – 2710 kcal
15 – 18 years	2160 kcal	1490 – 2710 kcal
19 – 30 years	2210 kcal	1970 – 2640 kcal

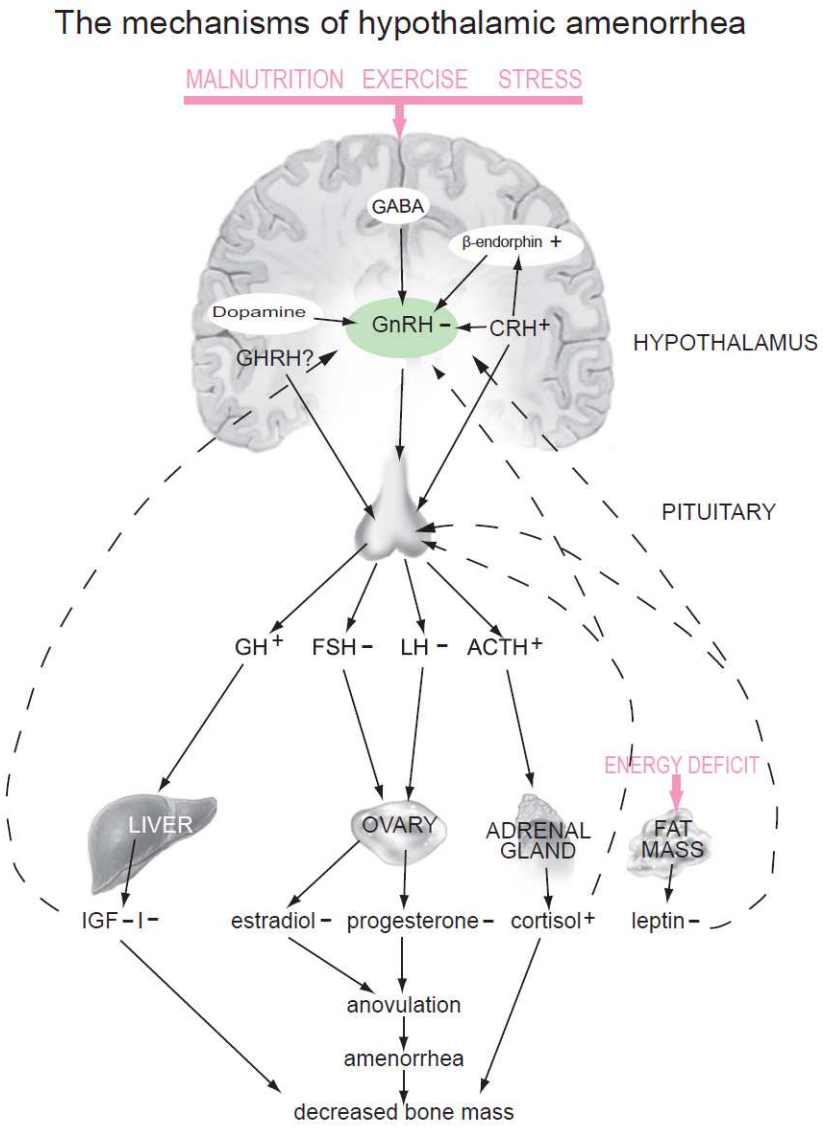


Figure 1. The mechanisms of hypothalamic amenorrhea and the clinical consequences. For explanations, see text. ACTH: adrenocorticotropic hormone; CRH: corticotropin-releasing hormone; FSH: follicle stimulating hormone; GABA: gamma amino butyric acid; GH: growth hormone; GHRH: growth hormone releasing hormone; IGF-I: insulin like growth factor I; LH: lutenizing hormone.

1.4.3 Eating disorders

Dieting and restricted caloric intake are very common among Swedish teenage girls (Schleimer 1983), some of whom are liable to be in the risk zone of an eating disorder. An instrument for diagnosing an eating disorder is the Diagnostic and Statistical Manual of Eating Disorders (DSM-IV) (APA 1994). The criteria are shown in Table 3. The prevalence of anorexia nervosa among Swedish teenage girls is reported to be 1% (Råstam et al. 1989). Population-based studies (Fairburn & Beglin 1990, Makino et al. 2004) put the prevalence of bulimia nervosa at 2-4% and of eating disorders not otherwise specified (EDNOS) at about 4-5%.

Among dieting schoolgirls, menstrual disorders have been found in 2.7% (Schleimer 1983). One of the diagnostic criteria for the diagnosis anorexia nervosa is amenorrhea. In anorexia nervosa, the usual mechanism of menstrual disturbance is hypothalamic amenorrhea due to starvation (Golden & Schenker 1994, Chan & Mantzorors 2005). Despite maintenance of normal body weight, menstrual dysfunction has been identified in 7-40% of women with bulimic eating disorders (Fairburn & Cooper 1982, Copeland & Herzog 1987, Copeland et al. 1995, Gendall et al. 2000, Crow et al. 2002, Naessén et al. 2006 a). Various mechanisms may interact in the etiology of menstrual disturbances in bulimia. The temporary starvation periods associated with bulimia may be responsible for the hypothalamic inhibition of the HPG-axis, resulting in low levels of FSH and LH (Schweiger et al. 1992, Cotrufo et al. 2000); low levels of thyroid hormones have also been reported (Schweiger et al 1992, Altemus et al 1996, Gendall et al 2000, Naessén et al 2006 a). Several studies indicate a link between bulimic eating disorder and increased occurrence of polycystic ovary syndrome (PCOS) (McCluskey et al. 1991, McCluskey et al. 1992, Jahanfar et al. 1995, Morgan et al. 2002, Hirschberg et al. 2004, Moran & Norman 2004, a et al. 2006). This suggestion is based on an increased frequency 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 and hirsutism (Sundblad et al. 1994, Cotrufo et al. 2000, Monteleone et al. 2001, Naessén et al. 2006 a) in bulimic women.

Table 3. Criteria of eating disorders according to the Diagnostic and Statistical Manual of Eating Disorders – 4th edition (DSM-IV)

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)
- 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 behavior

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

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 anorexia nervosa

Subtypes

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

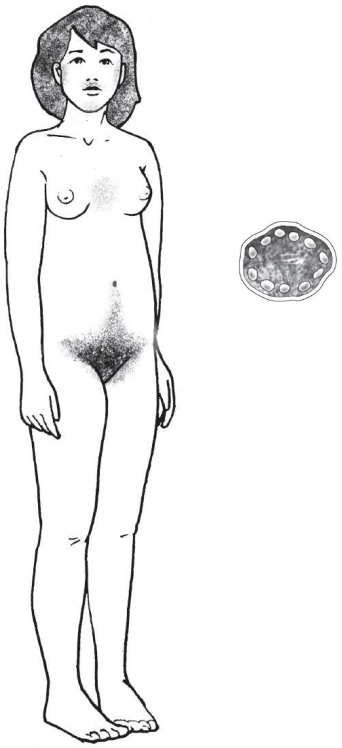
Non-purging type: The person uses other inappropriate compensatory behaviors, such as fasting or excessive exercise

Eating disorders not otherwise specified (EDNOS)

- All of the criteria for anorexia nervosa are met except that the individual has regular menses
- All the criteria for anorexia nervosa are met except that, despite significant weight loss, the individual's current weight is in the normal range
- All the criteria for bulimia nervosa 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 for bulimia nervosa.

1.5 POLYCYSTIC OVARY SYNDROME

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, Diamanti-Kandarakis 2008). The syndrome is furthermore associated with insulin resistance and abdominal obesity (Essah et al. 2007) (Figure 2). Several factors seem to interact in the development of PCOS. A genetic predisposition is required (Carey et al. 1993, Franks et al. 1997, Moran & Norman 2004), though there is also a link with environmental factors. Disturbed appetite regulation, with an increased craving for carbohydrates, decreased sense of satiation and an increased occurrence of binge eating, has been reported in women diagnosed with PCOS (Hirschberg et al. 2004, Moran & Norman 2004). However, there are also indications of improved physical performance in women diagnosed with PCOS, as well as protection from the metabolic consequences of estrogen deficiency (Rickenlund et al. 2003).



- **Oligomenorrhea/ secondary amenorrhea**
- **Polycystic ovaries**
- **Decreased fertility**
- **Hirsutism**
- **Acne**
- **Obesity, abdominal**

Figure 2. Characteristic clinical symptoms of PCOS.

The definition of PCOS has changed over time. The criteria for the diagnosis of PCOS were revised at the 2003 Rotterdam consensus workshop sponsored by the European Society of Reproductive Medicine (ESHRE) and the American Society for Reproductive Medicine (ASRM) (The 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.

Oligomenorrhea or menstrual irregularities in adolescents may be an early sign of PCOS rather than a stage in the physiological maturation of the HPG-axis. The diagnostic criteria of PCOS for teenagers are suggested to differ somewhat from those for adult women (Rosenfield et al. 2000): 1. oligo/amenorrhea (≥2years), 2. clinical signs of hyperandrogenism (severe acne 2-3 years after menarche or late debut of acne or hirsutism), 3. biochemical signs of hyperandrogenism. A combination of two of these three criteria is thought to be sufficient for diagnosis. The ultrasound picture of PCO is not included because ultrasound could be difficult to perform in teenagers who have not had their first intercourse.

1.6 BONE MASS

The bone matrix consists of 35% organic and 65% inorganic components (Prince et al 2000). Bone is categorized into two types, trabecular and cortical. The spine consists of 62-70% and the femoral neck of 25% trabecular bone (Riggs et al. 1982, Snow-Harter & Marcus 1991, Jee 2001). Cortical bone, e.g. in the femoral shaft, has a higher density than trabecular, which has a larger surface area per unit volume (Prince et al 2000). Bone is a metabolically active organ. Remodelling, resorption and formation of bone occur on the surface (Speroff et al. 2005 c). Because of trabecular bone's larger surface area, its bone remodelling frequency is about five to tenfold higher than that of cortical bone (Prince et al. 2000) and it is thus more apt to reflect early changes in bone homeostasis.

During adolescence, an increase in bone mineral density (BMD) is tied to pubertal progression. The increase in girls slows down after menarche. By the age of 12 years, girls have accumulated slightly more than 83% of the adult body's BMD (Lloyd et al.1992). Within a year after attaining menarche, lumbar spine BMD in girls is 71% of the adult level. One or two years post menarche, lumbar spine BMD is 95% of the adult level, which is reached 2-4 years post menarche (Bonjour et al. 1991, Abrams et al. 1996), although some further increase can occur up to the age of 20 years (Thomas et al. 1991). In the hip, peak BMD appears to be reached before the termination of longitudinal growth; increments slow down dramatically around 16 years of age but can continue up to the age of 20 years (Thomas et al. 1991). Trabecular bone seems to reach peak values at around 20 years of age (Bonjour et al 1991). A low peak bone mass attained during adolescence and young adulthood is associated with osteoporosis later in life and an increased risk of fracture (Recker et al 1992, Heaney et al 2000). Bone mass development during adolescence in females and males can be seen in Figure 3.

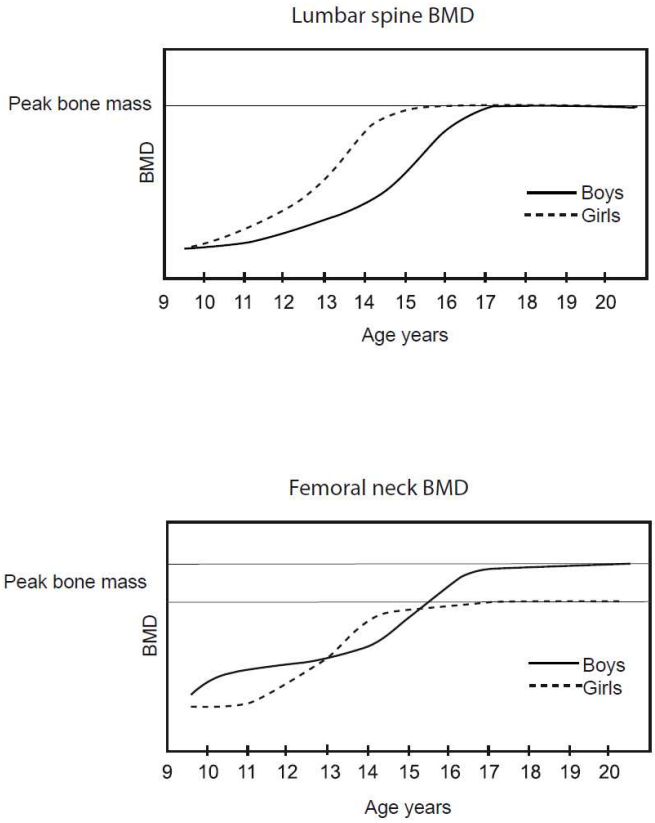


Figure 3. BMD in the lumbar spine and femoral neck in adolescent girls and boys. Adopted from Bonjour et al. 1991.

Several factors are known to influence bone mass in young women. Besides hereditary factors (Krall & Dawson-Hughes 1993, Geugen et al. 1995), BMD has been positively correlated to body weight and physical activity, whereas amenorrhea, low weight and eating disorders have a negative influence (Pollitzer & Anderson 1989, Matkovic et al 1990, Borer 2005, Naessén et al 2006 b, Havill et al. 2007, Legroux-Gérot et al. 2007, Rautava et al. 2007, Karlsson et al. 2008). The increment in BMD relative to bone mineral content (BMC, g; BMD/BMC) in girls is particularly pronounced over a 3-year period from 11-14 years of age.

Estrogen is important for maintenance of bone mass. The mechanisms involve specific estrogen receptor actions regulating bone remodeling, resorption and formation (Riggs et al 2002, Syed & Khosla 2005). Suppression of osteoclastic bone resorption and stimulation of osteoblastic bone formation are considered to form the basis for bone-preserving effects of estradiol (Nilsson et al 2001, Manolagas et al 2002, Syed & Khosla 2005). Long-standing amenorrhea and estrogen deficiency will lead to a rapid loss of bone mass, particularly of trabecular bone, such as in the spine (Biller et al.1991, De Souza & Williams 2004). Limited data suggest that low dose oral contraceptives counteract bone loss in young women (Warren et al. 2005), though other studies found no beneficial effect (Grinspoon et al. 2003). In conditions where

suppression of bone formation predominates, such as anorexia nervosa, oral contraceptives seem to have little effect (Grinspoon et al. 2000, Strokosch et al. 2006).

Adolescents with menstrual disturbances and PCO have been found to have a higher BMD than those with normal ovaries (To & Wong 2005). Hirsute women have demonstrated significantly higher BMD levels than controls (Glintborg et al 2005). Douchi and co-workers conclude that in women with PCOS, testosterone influences regional BMD by increasing regional muscle mass. Furthermore, free testosterone has been found to correlate positively with neck and hip BMD levels independently of BMI in premenopausal women (Douchi et al. 2001, Glintborg et al 2005).

Nutritional status is another essential factor for bone health. Cross-sectional studies in premenopausal women (Van Loan & Keim 2000, McLean et al. 2001 a, b) have shown that marked dietary restraint among women with normal body weight has a negative influence on BMC and possibly also on BMD (McLean et al 2001 a,b). Apart from insufficient food intake, dieting may also be associated with psychological stress caused by a constant focus on food (McLean et al 2001 a, b). Sleep quality may also be important for bone health (Elgan & Friedlund 2006). Low BMI, energy deficiency and eating disorders are well-known factors associated with low BMD, osteoporosis and an increased risk of fractures (Wolfert & Mehler 2002, Bass et al. 2005, Borer 2005, Naessén et al. 2006 b, Sööt et al. 2006, Havill et al. 2007, Miyabara et al. 2007, Rautava et al 2007, Karlsson et al. 2008). The low BMD in these women is due to decreased bone formation in addition to increased bone resorption (Soyka et al. 1999, Legroux-Gérot et al. 2005, Vescovi et al. 2008).

In anorexia nervosa, up to 50% of anorectic patients have been found to have osteoporosis in the lumbar spine (Legroux-Gérot et al. 2005). Recovery from anorexia nervosa will not always compensate for an early bone loss (Zipfel et al. 2001, Soyka et al. 2002, Wentz et al. 2003). Studies of BMD in women with other kinds of eating disorder have produced conflicting results, showing normal (Newman & Halmi 1989, Sundgot –Borgen et al. 1998, Zipfel 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). However, it is well known that 25-30% of patients with bulimia have a previous story of anorexia nervosa (Fairburn & Hope 1988, Kaye et al. 2000). Naessén and co-workers have demonstrated that low BMD in bulimic women could be explained by previous anorexia nervosa, and that bulimia as such is not associated with low bone mass (Naessén et al. 2006 b).

The associations between BMD and physical activity are well known and supported by numerous reports (Bass et al. 2005, Borer 2005, Sööt et al. 2006, Havill et al. 2007, Miyabara et al. 2007, Rautava et al. 2007, Karlsson et al. 2008). Mechanical loading of increasing intensity and frequency, promotes the formation of bone (Turner 1998, Meyer et al. 2004). In adults, a high lean body mass (LBM) is also related to high BMD (Winters & Snow 2000), since with increasing LBM, the skeleton has to adapt to supporting a larger and stronger muscle mass. Therefore, physical activity and sports, which stimulate muscle growth and expose the skeleton to mechanical forces, promote a high bone density (Madsen et al. 1998, Kohrt 2001, Meyer et al. 2004). In adolescent girls, however, fat mass has been shown to be more strongly associated with bone mass than lean body mass (Hage et al. 2008).

Low bone mass is common in athletes with amenorrhea (Warren 1980, Drinkwater et al. 1984). The reason is considered to be combined effects of low energy availability

and its endocrinal consequences (De Souza & Williams 2005), which include hypoestrogenism, hypercortisolism, with catabolic effects on the skeleton and low levels of anabolic hormone IGF-I (Bennell et al. 1997, Otis et al. 1997, De Souza & Williams 2004, Gibson et al. 2004, Tauchmanova et al. 2007). The main effect will be on trabecular bone, due to the higher turnover of this tissue compared to the cortical skeleton. However, in women athletes in whom menstrual disturbances are caused by hyperandrogenism, the risk of a decline in BMD should be lower, since testosterone has anabolic effects on the skeleton (Notelovitz 2002). This is supported by the study by Rickenlund and co-worker, who reported a similar BMD in hyperandrogenic athletes with menstrual dysfunction compared to regularly menstruating athletes, and higher values than those of normoandrogenic women with oligo/amenorrhea (Rickenlund et al. 2003).

1.7 LIFE QUALITY AND LIFE STYLE

The definition of good health according to WHO (WHO conference 1978) is a state of complete physical, mental and social wellbeing and not merely the absence of diseases or infirmity. Health could also be described in terms of quality of life. Positive variables indicating a high quality of life could be happiness and positive thinking about the future, a feeling of being alert and rested, being content with life and body and doing well at school or work. Negative variables indicating a low quality of life could be sadness, negative stress, depression, suicidal thought or somatic symptoms like pain in the stomach (Forsell & Dalman 2004).

Quality of life is influenced by lifestyle factors. However, universally applicable guidelines are not possible because a particular life style can be positive for one person and negative for another. Furthermore, a way of life may be favorable for one parameter and unfavorable for another. Smoking, for example, is known to have adverse effects on health but could improve the feeling of being at ease in a social context. Experimenting with different life styles during adolescence is a way to find out what is optimal for one's quality of life.

The concept of life style includes numerous different habits, for instance eating and sleeping habits, psychical exercise, school/work attendance, sexual habits, general safety (e.g. using a helmet when riding a bike or a safety-belt in a car), and drug use. The school health department in Stockholm publishes health surveys on a regular basis. One such profile is constructed for teenagers aged 14-15 years and another about 2 years later. In these surveys, questions about family support, physical activity and eating and sleeping habits are combined with questions about physical and psychological wellbeing (Bråkenhielm 2001-2007). In recent decades an increase in overweight or obesity among teenagers has been noticed and a twofold increase in BMI > 2 standard deviations (SD) has been reported by the school health system (Bråkenhielm 2007). Overweight, food and physical activity are closely related. Interventions against overweight and obesity are a responsibility for society as a whole (SBU 2004). However, interventions at school could include the provision of suitable food and increased physical activity for major groups of adolescents in their upper teens. Low physical activity in many school programs may be a great disadvantage for adolescents' future health if it is not made up for by physical activity in their spare time (Bråkenhielm 2005, 2007). Several other reports on the health behavior of schoolchildren aged 13-15 years have been published during the last decades (Marklund 1997, HBSC 2004). These, like the reports from the school health system,

indicate that most teenagers are satisfied with their family and life and have a life style that is likely to be beneficial for their health. At the same time, there is a small group of teenagers who do have problems; many of them have a cluster of problems as well as a hazardous life style. Alcohol and drug use may be an indication as well as a cause of an unhealthy life. Furthermore, in studies by Andersson-Ellström and co-workers, the degree of sexual experience was found to be strongly related to life style factors such as smoking and alcohol use (Andersson-Ellström et al. 1996). National data are collected annually on drug and alcohol use. An increase has been noticed during the last decade (CAN 2004).

During the period when the adolescent navigates towards adulthood by trying different life styles, peers become increasingly important and their life styles will therefore be mimicked. The teenager will try what is the good but also what is not so good. The support and guidance of the family will be important for a positive outcome. During adolescence, several problems may be related to life style. However, self-reported health problems and diseases may differ significantly between teenagers' and adults' health profiles according to traditional diagnoses.

The National Board of Health and Welfare in Sweden has reported on the role of youth clinics, with their wide variety of professions, in supporting young people (Socialstyrelsen 2005, 2008 a, b).

2 AIMS

The general aim of this thesis was to investigate aspects of reproductive health in adolescent girls, for example menstrual disorders, eating behavior and long-term medical consequences, including bone mass. Furthermore, the aim was to study sexuality and social factors in adolescent girls in comparison with boys.

The specific aims of the thesis were:

- To investigate endocrine mechanisms of menstrual disturbances and associated factors among adolescent girls visiting a Swedish youth clinic.
- To perform a clinical follow-up of menstrual status and eating behavior in adult women who had had secondary amenorrhea or oligomenorrhea in adolescence.
- To evaluate long-term effects on bone health in women diagnosed with menstrual disorders in adolescence.
- To investigate family circumstances and aspects of life quality and sexuality in girls and boys visiting a youth clinic.

3 MATERIALS AND METHODS

3.1 ADOLESCENT STUDY (I)

3.1.1 Subjects

The study was performed at a youth clinic, Stockholms Skolors Ungdomsmottagning, that is a part of the school health system. The services are available up to the age of 20 for girls and boys living in Stockholm County, and up to 23 for those living in the city proper. The regular staff at the clinic consists of one gynecologist, three midwives, one psychologist, two social guidance counselors and one dietician. Furthermore, four consultant physicians regularly work at the clinic: a pediatrician, a venereologist, an andrologist and a psychiatrist specialized in pediatrics and adolescece. To visit the clinic, young people make an appointment either themselves, through their parents or by a referral from the doctor or nurse at their school.

During a period of five years, about 3000 new appointments for girls were made with the same gynecologist (principal author) at the youth clinic. The reason for 215 of these consultations was a menstrual disorder. Other common reasons were dysmenorrhea, contraceptive advice or counseling on sexually transmitted infections. The girls were often referred to the gynecologist by midwives at the youth clinic or by school doctors. There were 203 girls who made an appointment for a menstrual disorder of varying duration. Girls with primary amenorrhea (n = 12) and those with a positive pregnancy test were not included in this study.

The clinical records of the 203 girls with secondary menstrual disorders were retrospectively reviewed and form the basis of this study. Secondary amenorrhea was defined as no menstrual periods for more than 6 months. Oligomenorrhea was defined as a menstruation interval of 6 to 24 weeks with a maximum of 4 menstruations during a 6-month period.

The study was approved by the Research Ethical Committee of the Karolinska Institutet.

3.1.2 Study design and investigational setting

The gynecologist took a detailed history in a standardized manner of previous health problems, earlier menstrual pattern, present somatic problems, weight loss, family situation, social life, school situation, eating habits, physical activity and psychological problems. The growth charts of the girls were requested from the school health clinics. Experience of weight loss was obtained by history and/or from the growth chart. Gynecological examination and a vaginal ultrasound were performed in girls who had previous experience of sexual intercourse. About 50% of the girls were virgins and they were examined by external inspection of the genitalia and an abdominal ultrasound.

A blood sample was collected for hormonal measurements of gonadotropins (LH, FSH), thyroid stimulating hormone (TSH), free thyroxin (T4), prolactin, testosterone and sex hormone-binding globulin (SHBG).

PCOS was diagnosed according to the Rotterdam consensus, based on two of the following three criteria: 1. oligo- or anovulation, 2. clinical and/or biochemical signs of hyperandrogenism, and 3. PCO on ultrasound (3).

Evaluation of the pituitary was done if the girl had a secondary amenorrhea lasting more than one year. Investigation of the sella turcica was made by x-ray during the first 2-3 years of the study and thereafter by Magnetic Resonance Imaging (MRI). In the girls with more than six months of secondary amenorrhea, estrogen status was evaluated by a gestagen test (10 mg medroxyprogesterone for 10 days).

Evaluation of eating habits and diagnosing of eating disorders were performed by the gynecologist in collaboration with the dietician and the psychologist at the youth clinic. One third of the girls met the dietician and/or the psychologist, either if they had expressed a wish to do so or if something in their history justified this. The dietician examined the girls' eating habits, dietary composition, number of meals per day, portion sizes and total daily energy consumption, and also noted the use of laxatives, vomiting and excessive compulsive eating. The psychologist evaluated psychological and social problems, in addition to the eating behavior, and offered a therapeutic contact if needed. Eating disorders were diagnosed according to DSM-IV (APA 1994).

The level of physical activity was evaluated using the classification of the energy cost of human physical activities developed by Ainsworth et al. (2000). One point corresponds to an energy expense of 1 kcal/minute. Each type of physical activity includes a scale of points that correspond to how vigorously the sport is practiced. The mean value for the specific sport was used and was multiplied by the number of hours per week the specific sport was practiced. If more than one sport was practiced, the total sum is given. Smoking was defined as daily smoking of one or more cigarettes. Regular use of alcohol was defined as a regular intake of alcohol several times a month, and high consumption as being intoxicated on a regular basis.

The evaluation period was three months and included a follow-up visit to the gynecologist.

3.2 FOLLOW-UP IN ADULTHOOD (II)

3.2.1 Subjects

All the subjects who took part in the adolescent study (I) – 117 girls diagnosed with secondary amenorrhea and 86 girls with oligomenorrhea – were contacted by letter about six years after the primary evaluation and invited to take part in the follow-up study. The follow-up was performed at the same youth clinic as the adolescent study (I).

Informed consent was obtained from all subjects before participation. The study was approved by the Research Ethical Committee of the Karolinska Institutet.

3.2.2 Study design and investigational setting

The same gynecologist (MWA) as in the original investigation took a detailed history of menstrual pattern and a structured interview regarding somatic problems, weight loss, physical activity and psychological problems. Definitions of secondary amnorrhea and oligomenorrhea were the same as in the Adolescent study (I). Gynecological examination was performed, women having menstrual disturbance also underwent vaginal ultrasound and a blood sample was collected for hormonal measurements.

All women underwent evaluation of eating habits and diagnosis of eating disorders was performed by the dietician in collaboration with the gynecologist at the youth clinic. The dietician recorded dietary composition, number of meals per day and portion sizes, using a 24-hour recall. The results were related to the Swedish nutrition recommendations. The dietician also investigated weight control behavior, such as restrictive eating, use of laxatives and vomiting, and excessive compulsive eating, using a structured interview. Eating disorders were diagnosed according to DSM-IV (APA 1994). Recovery from eating disorders was defined as not meeting diagnostic criteria for any eating disorder.

As an additional instrument to evaluate women in the risk zone of eating disorders, the Eating Disorder Inventory-2 test (EDI-2) (Garner 1991) was used. EDI-2 is a widely-used test for eating disorders. The original version of EDI provides standardized subscales on eight dimensions: drive for thinness, bulimia, body dissatisfaction, ineffectiveness, perfectionism, interpersonal distrust, interoceptive awareness, maturity fears (Garner et al. 1984). Normative information for the Swedish population of students and patients is also available (Norring & Sohlberg, 1988). EDI-2 contains three additional dimensions: asceticism, impulse regulation and social insecurity (Garner 1991). At the follow-up in adulthood, an EDI-2 test with the eleven defined EDI-scores at risk for eating disorders was used. The EDI-2 background data was also collected, giving the history of weight-control habits. These responses were used in the evaluation of an eating disorder.

The level of physical activity was evaluated as in the Adolescent study (I), using the classification of energy cost of human physical activities developed by Ainsworth et al (2000).

3.3 BONE MINERAL DENSITY AT FOLLOW-UP (III)

3.3.1 Subjects, study design and investigational setting

The subjects and the study design were the same as in the Follow-up study in adulthood (II).

Informed consent was obtained from all subjects before participation. The study was approved by the Research Ethical Committee of the Karolinska Institutet.

3.3.2 Bone mineral density

BMD and body composition were determined by dual-energy X-ray absorptiometry (DXA), using a Hologic QDR ® 4500A scanner (Hologic, Inc., Bedford, MA, USA). The examination was a whole-body scan. In this scan, BMD was determined for the lumbar spine (L1 - L4), the left femoral neck and the pelvis (Figure 4). Low BMD in the femoral neck or lumbar spine was defined as osteopenia if the T-score was between -1 and -2.5 SD of that for young adults (peak bone mass) and as osteoporosis if the T-score was below -2.5 SD, according to the World Health Organization (WHO). The age-adjusted Z-score was also used for evaluation. The T- and Z-scores were estimated using mean BMD and SD values supplied by the Hologic equipment manufacturer. The reproducibility of the whole body BMD is calculated as <0.01 g/cm²or 0.1 SD (Nuti et al.1991, Brismar & Ringertz 1996).

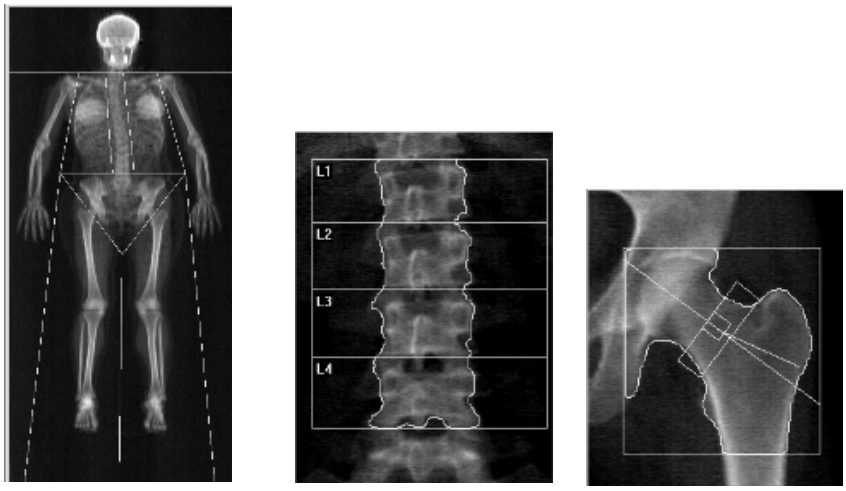


Figure 4. DXA scans of the whole body, the lumbar spine and the femoral neck.

3.4 ANALYTICAL METHODS

Laboratory analyses of pituitary, gonadal, adrenal and thyroid hormones were performed at the Department of Clinical Chemistry, Karolinska University Hospital. Methods, manufactures and detection limits are presented in Table 4. The ratio between total testosterone and SHBG was used as an index of biologically active testosterone. The upper reference limit for the testosterone/SHBG ratio at our department is 0.050. A testosterone/SHBG ratio >0.050 and a LH/FSH ratio > 2 were classified as biochemical hyperandrogenism. LH values <2 U/L were considered to indicate hypothalamic inhibition of the HPG-axis.

Table 4. Method principles, manufacturers and method prestanda for endocrine assays used in the present study.

Analyte	**Method**	**Manufacturer**	**Detection limit**	**Within assay CV**	**Between assay CV**
FSH	TRFIA	Perkin-Elmer	0.05 U/L	2.0%	3.0%
LH	TRFIA	Perkin-Elmer	0.05 U/L	1.7%	2.0%
T, paper I	RIA	Siemens	0.1 nmol/L	6.0%	10.0%
T, paper II	TRFIA	Perkin-Elmer	0.3 nmol/L	2.1%	6.1%
SHBG	TRFIA	Perkin-Elmer	0.5 nmol/L	1.3%	1.9%
PRL	TRFIA	Perkin-Elmer	0.04µg/L	1.9%	3.2%
TSH	TRFIA	Perkin-Elmer	0.005 mU/L	3.0%	5.0%
fT4	TRFIA	Perkin-Elmer	2 pmol/L	5.0%	4.0%
A-4	RIA	DiaSorin	0.1 nmol/L	6.9%	10.7%
17-OHP	RIA	CIS	0.1 nmol/L	7.8%	10.0%
DHEAS	CIA	Nichols	0.03 µmol/L	5.4%	9.1%

FSH, follicle-stimulating hormone; LH, luteinizing hormone; T, testosterone; SHBG, sex hormone-binding globulin; PRL, prolactin; TSH, thyroid-stimulating hormone; fT4, free thyroxine; A-4, androstenedione; 17-OHP, 17-hydroxyprogesterone; DHEAS, dehydroepiandrosterone sulfate. Units for protein/peptide hormones: *FSH:* 2:nd IRP 78/549. *LH:* 2:nd IRP 80/552. *Prolactin:* 3:rd IRP 84/500. *TSH:* 2:nd IRP 86/558.

TRFIA = time resolved fluorescence immunoassay, RIA = radioimmunoassay, CIA = chemoluminiscence immunoassay. Manufacturers: *Perkin-Elmer:* Perkin-Elmer Finland OY (previously Wallac OY), Turku, Finland. *Siemens:* Siemens Medical Solutions Diagnostics (previously Diagnostic Products Corp.), Los Angeles, CA. *Dia-Sorin:* Dia-Sorin Inc., Greater Minneapolis – St Paul Area, Minnesota. *CIS:* CIS Bio International, Gif-sur-Yvette, France. *Nichols:* Nichols Institute Diagnostics, San Capistrano, CA.

3.5 LIFE QUALITY AND SEXUALITY (IV)

3.5.1 Subjects, study design and investigational setting

During a three-month period, all young people, 702 individuals (about 80% girls and 20% boys), visiting Stockholm Skolors Ungdomsmottagning were asked to fill in a questionnaire. The participants were either young people coming for counselling/treatment or partners and other accompanying persons in the same age group.

The study was approved by the Research Ethical Committee at Karolinska University Hospital, Stockholm.

3.5.2 Survey

The questionnaire was constructed to cover all aspects of the clinic's activities; all the professional groups represented at the clinic took part in its development. The questionnaire was designed in cooperation with the Research and Development Department at the City of Stockholm Education Office. One extra person was employed to present the survey and collect the completed questionnaires. Answering was completely voluntary and the anonymity of the respondents was guaranteed. No names were registered, only age and sex.

Cross-sectional quantitative studies are frequently used to obtain data from a selected population, e.g. health surveys of a specific age group at school or visitors to a youth clinic. The main advantage of these surveys is the large amount of data in different areas that can be collected, analysed and compared with each other. To reduce the disadvantages, a draft questionnaire was used as a pilot so that changes could be made to avoid misunderstanding of questions and reduce the number of missing answers. Furthermore, in order to minimize any tendency not to answer properly or to drop out before finishing the questionnaire, participants were allotted a private space that ensured anonymity and given plenty of time in which to fill in the questionnaire. One person was assigned just to administrate the survey and to emphasize how important it was that the participants completed the questionnaire.

Reliability can be determined as the extent to which the measuring procedure produces the same result in repeated trials. However, test – retest reliability was not possible in this survey because the responders were anonymous. Instead, a scale reliability test, assessed by calculating Cronbach's Coefficient Alpha, was used to test internal consistency. Alpha = 0.7 indicated good reliability. A low coefficient indicates low consistency between the group of questions but may also be due to the participant misunderstanding the question, or indicate a less serious attitude to answering.

Validity describes the extent to which the measuring procedure measures what it is intended to measure. To control external validity, the respondents' answers were compared to other reliable information, such as demographic or register data and or other validated surveys. One indication of high sample validity was the high response rate.

3.6 STATISTICAL ANALYSES

Statistica™ 6.1, 7.0 and 8.0 softwares (Statsoft® Inc., Tulsa, OK, USA) were used for analyzing the data. In all four studies (I-IV), normally distributed data are presented as an arithmetic mean and SD, otherwise as a median and range. For comparisons between groups, a t-test or the Mann-Whitney U-test was used for independent samples according to distribution. Pearson's Chi-square-test and the Fisher's exact test were used to analyze variables on a nominal scale.

In the Adolescent study (I), an analysis of covariance was performed to control for the effect of body weight on blood-pressure variables and pulse rate. Logistic regression analysis was used to evaluate the relationship between diagnosis (secondary amenorrhea/oligomenorrhea) and explanatory variables divided into blocks of endocrine variables and nutrition and life style variables. Family, school and social variables were not included due to many missing data. Stepwise logistic regression analyses were performed within each block and the significant variables from each analysis were then included in a final stepwise logistic model. For the stepwise selection we used a criterion for entry of a p-value <0.10 and for removal a p-value >0.10. The significance level was set at 0.05.

In the Follow-up study in adulthood (II), univariate logistic regression analysis was performed to evaluate the relationship between menstrual pattern at the follow-up and the following independent variables: weight loss, BMI, dietary intake, physical activity, eating disorders and PCOS diagnosis at the first visit. The same independent variables were used in the Follow-up study. A p-value <0.05 was considered statistically significant.

In the Bone mineral density at follow-up study (III), Kruskal-Wallis Anova was used for comparisons between groups of independent samples**.** Correlations were calculated with Pearson's product moment correlation coefficient. Forward stepwise multiple linear regression analysis was used to evaluate to what extent the variation in BMD measures could be explained by menstrual disorders, BMI, smoking, eating disorder and physical activities. For the stepwise selection we used a criterion for entry of a p-value <0.05 and for removal of a p-value > 0.05. Due to missing values for some of the independent variables, a subset of women was the basis for the determination of significant predictors. Then, for the final analyses, the chosen predictors were applied to the larger subset of women without missing values in these variables. For some variables with a positively skewed distribution, log-transformation was performed before the formal analyses. $P < 0.05$ was considered statistically significant.

In the Life quality and sexuality study (IV), scale reliability was assessed by calculating Cronbach's Coefficient Alpha.

4 RESULTS AND DISCUSSION

4.1 MENSTRUAL DISTURBANCES IN ADOLESCENCE

Menarche signifies that the final stage of puberty has been reached in healthy girls. The mean age of menarche in Sweden is 12.8 years (Lindgren et al. 1991). In the 203 girls participating in our study, the age of menarche averaged 13 years and the primary investigation was performed on average four years later (mean age 17.3 years). Irregular periods and anovulatory cycles are common during the years after menarche, with a prevalence of 85% in the first year, almost 60% during the second to third year, and about 25% in the sixth year after menarche (Read et al. 1984, Vuorurent & Huhtaniemi 1992). Many girls with anovulatory cycles and irregular periods present an endocrine picture that agrees with PCOS (Venturoli et al. 1995, van Hooff et al. 2000, Speroff et al. 2005 d). In our material, 54% of the girls had experienced regular cycles before the development of a menstrual disorder, i.e. secondary amenorrhea; the remaining 46 % had never had regular periods and could thus be at risk for PCOS.

4.1.1 Endocrine mechanisms

Among the girls with secondary amenorrhea, 36% had LH levels < 2, indicating a profound hypothalamic inhibition, i.e. hypothalamic amenorrhea, compared to 7% of the oligomenorrheic girls ($p<0.001$) (Figure 5). The hypothalamic inhibition was further supported by the finding that some 30% of the girls with secondary amenorrhea did not respond to the gestagen test, indicating low estrogen levels in this group. Amenorrheic girls with a history of weight loss had lower LH values than those without such a history ($p<0.001$). Hypothalamic amenorrhea is considered to be the most prevalent endocrine mechanism of amenorrhea among adolescents (Perkins et al. 2001, Golden & Carlson 2008).

The endocrine pattern of the oligomenorrheic group was characterized by a significantly higher frequency of LH/FSH ratio > 2 and a testosterone/SHBG ratio > 0.05 than the amenorrheic group ($p<0.001$ and $p<0.05$, respectively). Furthermore, a LH/FSH ratio >2 was found to be the strongest predictor of oligomenorrhea, with an odds ratio (OR) of 4.5 (95%confidence interval (CI) 2.0-11.1, $p<0.001$). The elevated ratio of testosterone/SHBG is considered to be a sensitive marker for androgen excess. There was a clear tendency for the occurrence of PCOS to be higher among the oligomenorrheic girls (55%) compared to those with secondary amenorrhea (38%) ($p=0.06$) (Figure 5).

The results from this study indicate that secondary amenorrhea and oligomenorrhea represent different hormonal patterns. A hormonal pattern in agreement with hypothalamic inhibition of the HPG-axis, which is most often an acquired functional disturbance, was more common in girls with secondary amenorrhea, whereas a pattern indicating hyperandrogenism dominated among oligomenorrheic girls.

As noted, the frequency of PCOS was high in both groups, which may be a consequence of the new diagnostic criteria also including a non-hyperandrogenic

phenotype of PCOS (Azziz 2005). However, the oligomenorrheic girls had to a great extent never experienced regular menstrual periods, which may support a genetic disposition for menstrual disorders, such as PCOS. Our results are in agreement with some previous studies suggesting that oligomenorrhea in young girls without obesity and obvious clinical symptoms of hyperandrogenism can be an early sign of PCOS (Siegberg et al. 1986, van Hooff et al. 1999, Avvad et al. 2001). Obesity in early puberty could also be a predisposing factor for the development of anovulatory cycles. However, only 12 of the girls with secondary amenorrhea and 13 of those with oligomenorrhea were overweight with a BMI >25.

Apart from hypothalamic amenorrhea and hyperandrogenism, we found no other specific endocrine disorders. The remainder of the girls are considered to have a dysregulation of the HPG-axis.

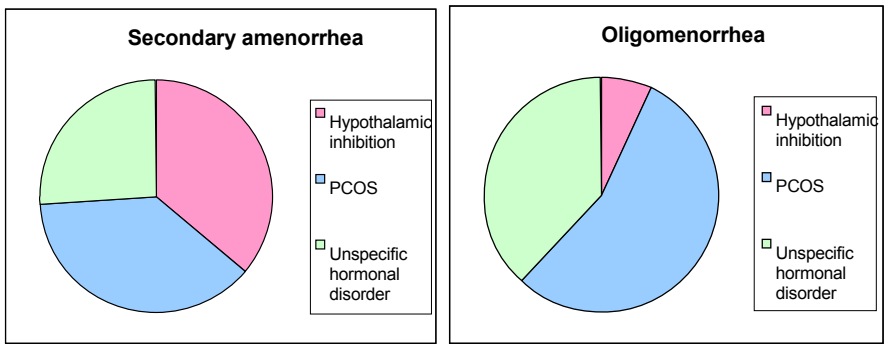


Figure 5. The proportion of hypothalamic inhibition, PCOS and unspecific endocrine disorder in girls with secondary amenorrhea or oligomenorrhea.

4.1.2 Eating behaviour in adolescence

A history of weight loss was common among the girls and significantly more frequent in the secondary amenorrheic group (62%) than in the oligomenorrheic group (36%), ($p<0.001$). This may indicate a more restrictive eating behavior and a higher energy deficiency in the girls with secondary amenorrhea. A growth chart and BMI typical for one of the girls with secondary amenorrhea and with a long history of restrictive eating can be seen in Figure 6.

Adolescents with a history of weight loss seemed to be at increased risk of an eating disorder. Thus, 83% of the amenorrheic girls and 61% of the oligomenorrheic girls with weight loss were diagnosed as having an eating disorder. Eating disorders were in general highly frequent in comparison with the estimated prevalences of anorexia nervosa 1% (Råstam et al. 1989), bulimia nervosa 2-4% and EDNOS 4-5% in young women (Fairburn & Beglin 1990, Makino et al. 2004). Eating disorders were significantly more common in the girls with secondary amenorrhea than in those with oligomenorrhea (68% vs 38%, $p<0.001$). A restrictive eating disorder (anorexia nervosa and EDNOS, partial anorexia nervosa) was particularly prevalent in the secondary amenorrheic group (39%) compared with the oligomenorrheic group (7%), ($p<0.001$).

Eating behavior among girls with secondary amenorrhea and oligomenorrhea in adolescence is shown in Figure 7.

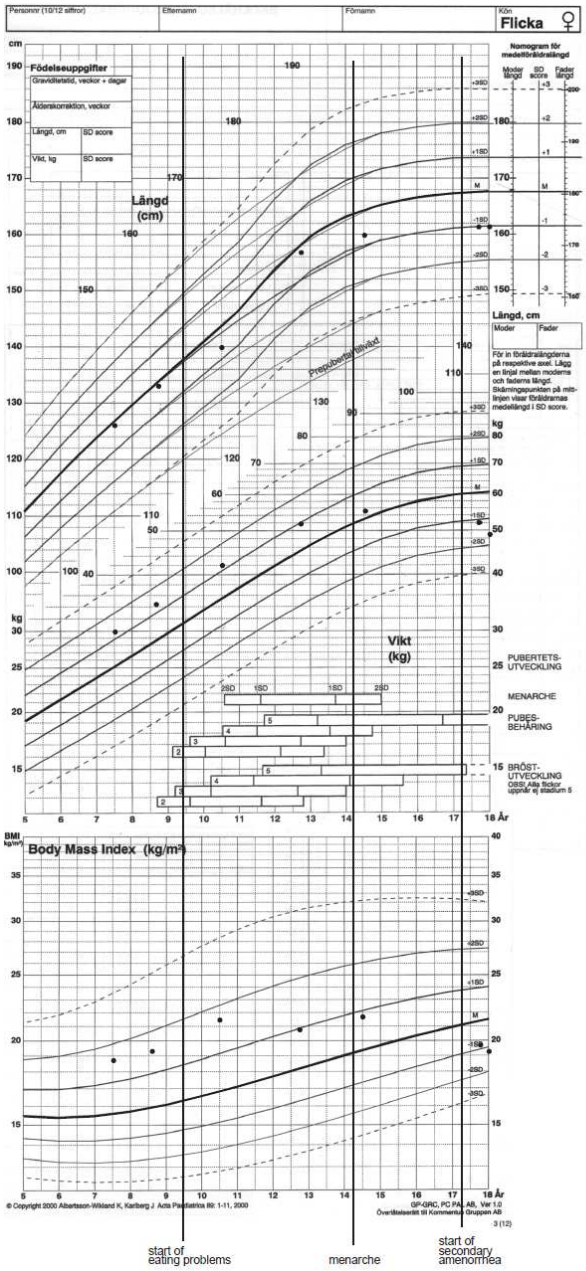


Figure 6. Growth chart of height, weight and BMI in a girl with secondary amenorrhea and restrictive eating disorder.

Eating disorder of restrictive type was found to be a strong predictor of secondary amenorrhea, with an OR of 4.4 (95%CI 1.3-15.1, $p<0.05$). Energy deficiency and eating disorders are well known to be associated with amenorrhea (Reindollar et al. 1981, Bachmann & Kemmann 1982, Reindollar et al. 1986, van Hooff et al. 1998). In anorexia nervosa, the usual mechanism of menstrual disturbances is functional hypothalamic amenorrhea due to starvation. However, the mechanisms behind disturbances in bulimic women are not fully understood. There are reports of low levels of gonadotropins, indicating hypothalamic inhibition of the HPG-axis also in bulimic women (Schweiger et al. 1992, Cotrufo et al. 2000). Furthermore, an association between bulimia nervosa and PCOS has been suggested (McCluskey et al. 1991, Raphael et al. 1995, Morgan et al. 2002, Naessén et al. 2006 a). In our study, a hyperandrogenic endocrine pattern and bulimic behavior in the oligomenorrheic group may support such an association.

4.1.3 Physical activity and social factors in adolescence

Physical activity outside school was significantly more common in the girls with secondary amenorrhea (56%) than in the oligomenorrheic girls (30%) ($p<0.001$). The degree of physical activity was also higher in the secondary amenorrheic group ($p<0.01$). Strenuous physical exercise, often in combination with dietary restriction, is known to inhibit the HPG-axis and to reduce LH pulsatility (Rickenlund et al. 2004). Although a few girls reported a high degree of physical activity, the total number of physically active girls was lower than the 74% reported in a questionnaire investigation among 9th-grade students in the Swedish compulsory school (Marklund 1997).

Family problems of a serious nature were reported by 42% of the girls with secondary amenorrhea and 53% of the girls with oligomenorrhea ($p<0.01$). Parents with alcohol and drug abuse were more frequent among the oligomenorrheic girls ($p<0.01$). The reported problems are far above the frequency of 5% noted in a report on the school population in Stockholm in 2001 (Bråkenhielm 2001). Own use of tobacco, alcohol and other drugs was much the same in the two groups of girls with secondary amenorrhea and oligomenorrhea, respectively, and in the same range as in other Swedish reports (Marklund 1997, Bråkenhielm 2002, CAN 2004). The girls in the oligomenorrheic group had more problems with friends than girls in the secondary amenorrheic group ($p<0.01$). It is suggested that the high frequency of stress factors among the adolescent girls in our study may be of importance for their menstrual dysfunction.

In conclusion, we found hypothalamic amenorrhea and hyperandrogenism to be common endocrine mechanisms of menstrual disorders in adolescent girls. Hypothalamic inhibition of the HPG-axis together with anorectic eating behavior dominated in the amenorrheic group, whereas a hyperandrogenic hormonal pattern, PCOS and eating disorders of bulimic type were frequent in the oligomenorrheic group. A high degree of family problems may also be of importance as general stress factors.

4.2 FOLLOW-UP OF MENSTRUAL DISORDERS/ EATING DISORDERS

After six years follow-up in adulthood (mean age 23.6 years), menstrual disturbance was still present in 62% of the subjects not using hormonal contraception. The frequency of menstrual dysfunction was significantly higher in the group with previous oligomenorrhea (76%) than in women with previous secondary amenorrhea (50%) ($p<0.05$). In contrast to the Adolescent study (I), most of the 34 women with menstrual disturbance at follow-up were oligomenorrheic and only six were amenorrheic. Twenty-four of the 34 women (70%) had never experienced regular menstrual periods since the first visit. The different rate of recovery from menstrual disorders in women previously diagnosed with secondary amenorrhea as compared to those with oligomenorrhea supports the notion of different mechanisms for menstrual dysfunction in these groups.

In the Adolescent study (I), hypothalamic inhibition of the gonadal axis dominated in the secondary amenorrheic group, whereas hyperandrogenism and PCOS were more common in the girls with oligomenorrhea. In the follow-up study, there was no indication of a severe hypothalamic inhibition among the women with menstrual disorders. In contrast, a hyperandrogenic endocrine pattern dominated and 60% of the women with persistent menstrual dysfunction were diagnosed with PCOS. Thus, our study is in agreement with the hypothesis that irregular menstruation during adolescence can be an early sign of PCOS (van Hooff et al. 1999). However, nutritional factors could also be of importance for menstrual disorders at follow-up since some of the subjects had low BMI and low levels of thyroid hormones.

The overall frequency of eating disorders at follow-up was still high (34%) (Figure 7) compared to what was seen at the first visit (53%), although about one-third of these girls had received specialist treatment for eating disorders. A similar frequency of persistent eating disorders has been observed in other follow-up studies (Fairburn et al. 2000, Herpetz et al. 2001, Råstam et al. 2003, Grilo et al. 2007). In contrast to the first visit, none of the women was diagnosed with anorexia nervosa or bulimia nervosa at the follow-up, but with EDNOS, indicating a less severe disease at follow-up. The rate of recovery from eating disorders differed significantly between the groups ($p<0.05$). In women with previous secondary amenorrhea, the occurrence of eating disorders was less than half of that at first visit, whereas the frequency in the group with previous oligomenorrhea was at the same level as at the first visit. The overall recovery from anorectic behavior was 23/24 (96%) and explained the decrease in eating disorder frequency among the women with previous secondary amenorrhea (Figure 7).

The logistic regression analysis revealed that previous anorectic eating disorder was the only significant predictor of a resumption of regular menstruations, with an OR of 5.3 (95%CI 1.4-19.5). Women with previous restrictive eating behavior also had a significant weight gain, from BMI 17.9 to 21.2. Although 19% had a diet with a low or extremely low fat content according to the Swedish nutrition recommendations, few women had an extremely low energy intake at follow-up. The results illustrate the well-known association between energy availability and activity of the HPG-axis. Results similar to those in our study were found in another long-term follow-up of functional hypothalamic amenorrhea by Falsetti et al. (2002). They demonstrated a recovery rate

of amenorrhea in about 70% of the women, and increased BMI was one important predictor of recovery.

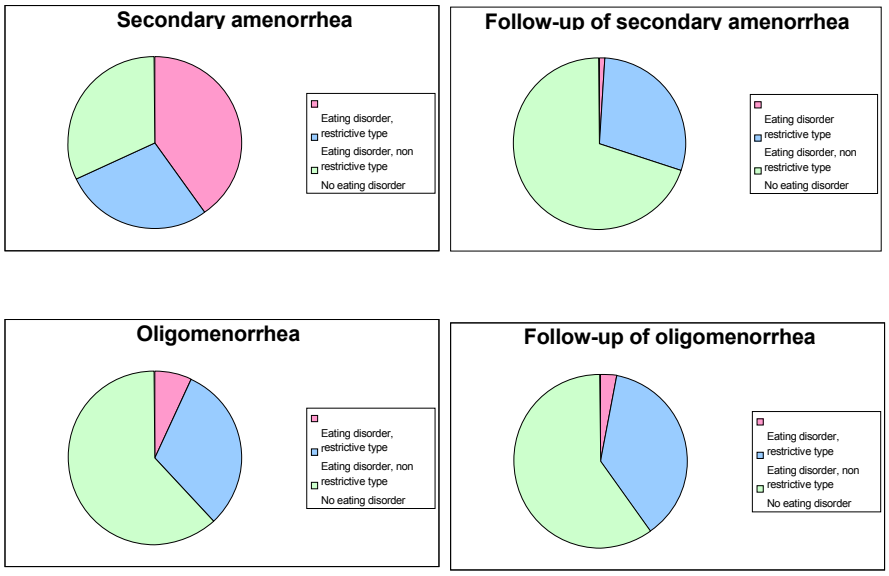


Figure 7. The proportion of eating disorder of restrictive type, eating disorder of non restrictive type and no eating disorder among girls with secondary amenorrhea or oligomenorrhea in adolescence and at follow-up.

We found no support for a relation between present menstrual disturbances and disturbed eating, since there were no differences in either weight control behavior, EDI at risk of eating behavior or diagnosis of eating disorder between women with and without menstrual disturbances. This may be explained by the very few subjects with anorectic eating disorders, since this type of eating disorder displays the strongest association with menstrual disturbance (I). Furthermore, anorectic behavior is primarily associated with secondary amenorrhea and not as clearly with oligomenorrhea, which was the most common dysfunction at follow-up. A lower frequency of EDI scores in the risk zone of eating disorders was found among the women using hormonal contraception compared to those who did not. This may indicate that the women using hormonal contraception had recovered from previous eating problems.

In conclusion, this prospective study of menstrual disturbances in adolescents has demonstrated that irregular menstruations and eating disorders are still frequent after six years of follow-up. PCOS was found to be the main mechanism explaining persistent menstrual disorders, whereas present eating disorder did not. However, recovery from anorectic eating disorders, which was most common in those with secondary amenorrhea, was a strong predictor of resumption of menses.

4.3 BONE MASS IN ADULTHOOD

Bone mineral density at follow-up was in general low, with an overall negative median Z-score of -0.46 SD in the lumbar spine and of -0.57 SD in the femoral neck. Women with previous secondary amenorrhea had significantly decreased BMD in the pelvis and lumbar spine in comparison with those with previous oligomenorrhea ($p < 0.05$). In women with secondary amenorrhea in adolescence, 53% had an osteopenia in the femoral neck or lumbar spine and 3 women had osteoporosis. The women with previous oligomenorrhea, had osteopenia in 41% and none of them had osteoporosis (Figure 8). There was no significant inter-group difference in fat per cent or in lean body mass.

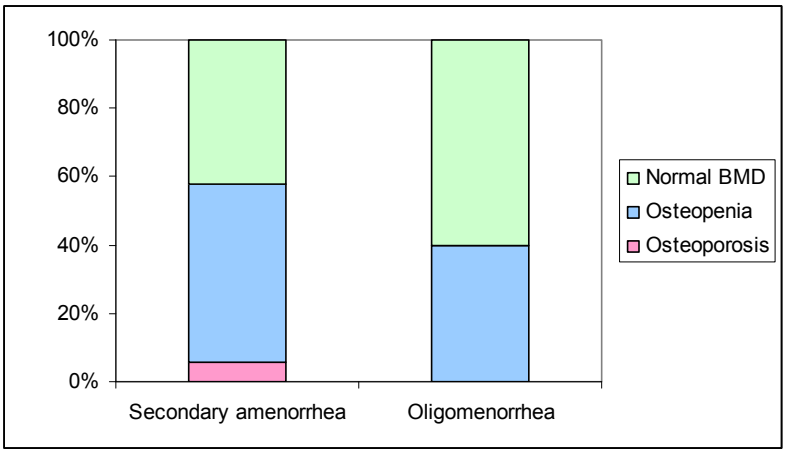


Figure 8. The frequency of normal bone mineral density (BMD), osteopenia and osteoporosis in adult women with secondary amenorrhea or oligomenorrhea in adolescence.

Comparison of BMD in groups of women with regular menstruation, menstrual dysfunction and hormonal contraception at follow-up, showed significantly higher frequency of osteopenia in the group of women with a regular menstrual pattern (70%) compared to those with menstrual dysfunction (35%) and hormonal contraception (47%) ($p < 0.05$, respectively).

A forward stepwise multiple linear regression analysis demonstrated that a restrictive eating disorder at first visit was a strong negative predictor of BMD. On the other hand, a minimum BMI ≥ 22, a high level of physical activity (medium intensity > 3 hours/week) and menstrual dysfunction at follow-up was a positive predictor of BMD. Figure 9 shows the frequency of osteopenia and osteoporosis in groups of women with restrictive eating disorder, EDNOS and normal eating behavior in adolescence.

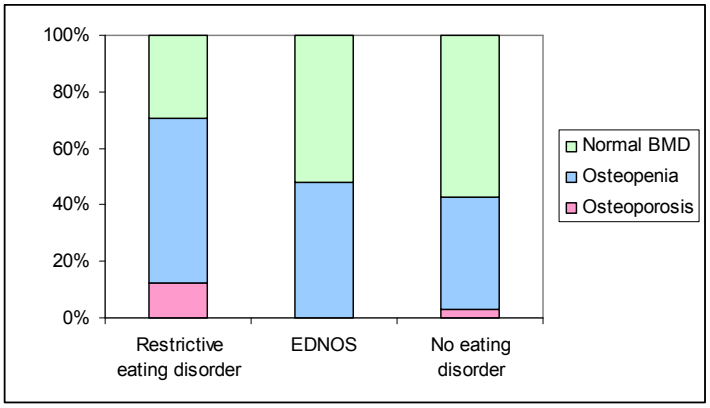


Figure 9. The frequency of normal bone mineral density (BMD), osteopenia and osteoporosis in adult women with restrictive eating disorder, eating disorders not otherwise specified (EDNOS) and normal eating behavior in adolescence.

It is well-known that restrictive eating behavior, as in anorexia nervosa, 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). Recovery from anorexia nervosa will not always compensate for an early bone loss (Zipfel et al. 2001, Soyka et al. 2002, Wentz et al. 2003). This was also shown in our study, where three women had osteoporosis at follow-up although they had recovered from their eating disturbances and only one still suffered from oligomenorrhea.

The women who recovered from anorectic behavior were also those with the highest rate of resumption of menses at follow-up, as shown in the follow-up in adulthood study (II). It is therefore likely that negative consequences of previous anorectic behavior explain the increased frequency of osteopenia in the group of women with regular menstruation at follow-up.

In the group of women with persistent menstrual dysfunction, 20 of 34 had PCOS and this subgroup had significantly higher total body BMD than the women with menstrual dysfunction of other etiology, such as hypothalamic amenorrhea ($p < 0.01$). PCOS is known to be associated with increased BMD, supporting that a hyperandrogenic disorder could protect from bone loss (Douchi et al. 2001, Yüksel et al. 2001, To & Wong 2005). Thus, previous anorectic behavior in the group of women with regular menstruation, as well as a high frequency of PCOS in the women with menstrual disorders, might explain the somewhat unexpected result of a significantly higher rate of osteopenia in the group of women with regular menstruation than in those with menstrual dysfunction or hormonal contraception at follow-up.

Limited data suggest that low dose oral contraceptives counteract bone loss in young women (Warren et al. 2005). However, there are also studies reporting no beneficial effect (Grinspoon et al. 2003). In conditions where suppression of bone formation predominates, such as in anorexia nervosa, oral contraceptives seem to have little effect

(Grinspoon et al. 2000, Strokosch et al. 2006). In this study, hormonal contraception had no significant influence on BMD.

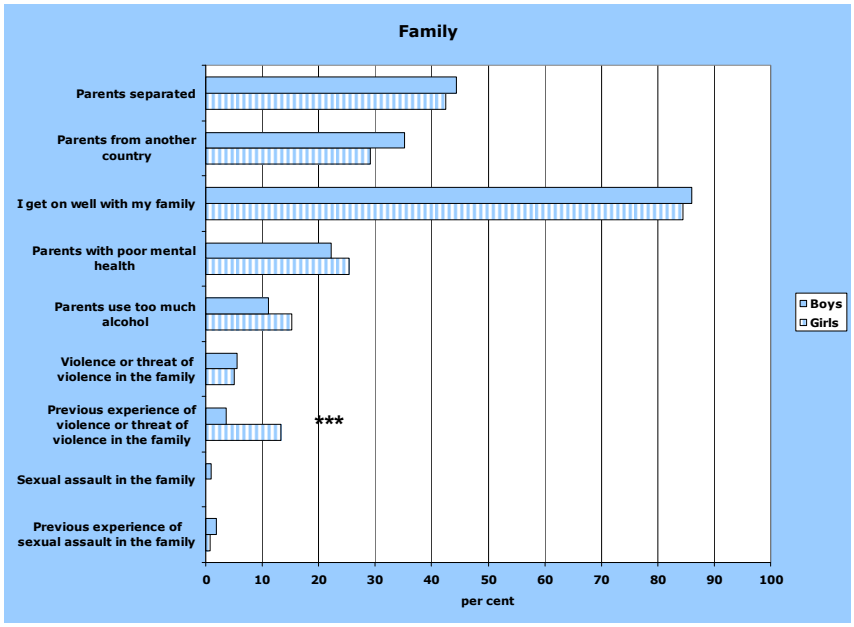
The positive associations between BMD and BMI or physical activity are supported by numerous reports (Bass et al. 2005, Borer 2005, Naessén et al. 2006 b, Sööt et al. 2006, Havill et al. 2007, Miyabara et al. 2007, Rautava et al. 2007, Karlsson et al. 2008). It has been demonstrated that fat mass is more strongly associated with bone mass than lean body mass in adolescent girls (Hage et al. 2008). However, in the present study there was no significant correlation between total lean body mass or fat mass and BMD.

In conclusion, a high frequency of osteopenia in the femoral neck or lumbar spine was found in adult women with menstrual disorders in adolescence. Previous anorectic behavior was the strongest negative predictor of BMD, whereas menstrual disorders as such had no negative influence on bone mass. The most important counteraction to low BMD was a high level of physical activity.

4.4 LIFE QUALITY AND SEXUALITY IN YOUNG PEOPLE

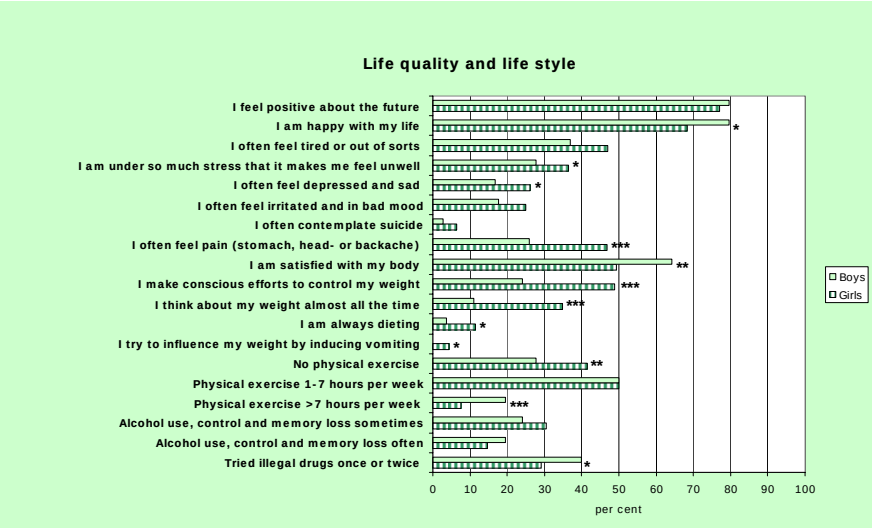
The survey was answered by 480 girls, age 18.7 ± 2.1 years, and 108 boys, age 19.8 ± 2.9 years. The gender distribution mirrors that of the clinic in that period. The overall response rate was 87%, with no difference between girls and boys.

Family situation is displayed in Figure 10. Most of the girls and boys got on well with their families. The responses to family items are in the same range as in the reports from Statistics in Sweden (SCB 2005). The higher frequency of previous experience of violence in the girls' families may indicate that girls visiting the youth clinic have a more problematic family background than boys. Some 20% of both girls and boys had parents with poor mental health. Such problems, including alcohol abuse among parents, were also found in the girls with menstrual disturbances in the Adolescent study (I), confirming the vulnerability of this group of girls.



Significant differences between groups are indicated; ***, $p < 0.001$.

Figure 10. Family situation in girls and boys.



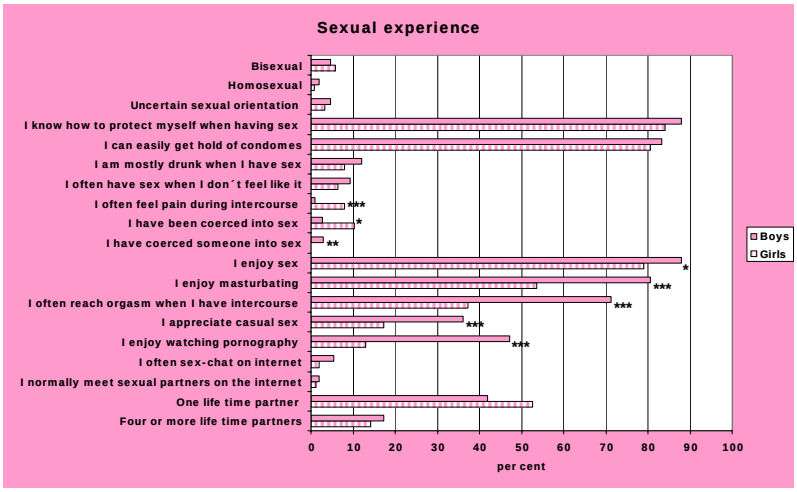
Significant differences between groups are indicated; *, $p < 0.05$; **', $p < 0.01$;***, $p<0.001$.

Figure 11. Life quality and life style in girls and boys.

The item "I am happy with my life" has been found to be an important indicator of problems (Bråkenhielm 2006); low scores are associated with other problems, such as depression, bullying and worries about the family. The figures in this study are in the same level as in other reports (SCB 2005, Bråkenhielm 2006). The boys scored significantly higher than the girls, which agree with the pattern in the reports above. The gender differences in some items were pronounced and could indicate that girls are more sensitive to their life situation.

Significantly more girls than boys were dissatisfied with their bodies, to judge from their overall responses to the items 'conscious efforts to control weight', 'thinking about weight', 'always dieting'. These results indicate a clear risk of developing an eating disorder.

The use of alcohol and illicit drugs among young people in Sweden has increased in recent decades (CAN 2004). The proportion of young people in this survey who are using alcohol to such an extent that they suffer memory loss that may incur negative medical and social effects seems to be slightly higher compared with other Swedish reports (SCB 2005, Socialstyrelsen 2005, Socialstyrelsen 2009). The use of illicit drugs is also higher than in other reports. These results from the larger unscreened group are comparable with those of the girls consulting for menstrual disturbances, Adolescent study (I).



Significant differences between groups are indicated; *, $p < 0.05$; **, $p < 0.01$; ***, $p<0.001$.

Figure 12. Sexual experience in girls and boys.

As these young people who voluntarily consult a youth clinic constitute a selected group with respect to sexual life, it is not possible to assess the external validity of our questionnaire in relation to other surveys, for example those performed in schools.

The proportion of the survey participants who had experienced sexual intercourse was the same (81%) among girls and boys. Age at first intercourse did not differ significantly between the girls and boys. The most common forms of contraception at the most recent intercourse were hormonal oral contraceptives, used by 30%, and condomes, 25%, while 20% had not used any method at all. Almost 10% reported being drunk when having sex. These figures are higher than in a country report for Sweden (Danielsson et al. 2001), indicating that a proportion of the clients at our youth clinic constitute a risk group for adverse reproductive health.

The responses about sexual orientation do not differ greatly from other reports (Novak & Karlsson 2006, Månsson et al. 2003). About 10% of the boys answered either "don't know" or did not respond to this item. They could be uncertain of their sexual orientation, but could also find the matter too embarrassing. The number of young people who had been coerced into sex was lower than in the study above by Novak and Karlsson (2006), but in the same range as in the survey by Edgardh and Ormstad (2000), though their definition excluded peer abuse. The higher proportion of girls than boys who had been coerced into sex agrees with the studies above. A significantly larger proportion of girls compared to boys were not enjoying their sexual life.

In conclusion, the results indicate a clear need for increased psychological and sexual counseling in this age group.

4.5 CLINICAL CONSIDERATIONS

The results of this thesis indicate that all young people visiting a youth clinic ought to be evaluated in a broad way to identify risk factors for adverse reproductive health and offered a possibility of prevention and/or treatment.

Menstrual disorders in adolescence could be related to physical, psychological as well as social factors. Eating disorders seem particularly important to diagnose. Evaluation of these problems in an adolescent is arduous. It is easy for a girl to consult for a menstrual problem but harder to realize and admit a disturbed eating behavior. Therefore a multi-professional team with gynecological, psychological and dietary competence will be more successful to diagnose the problem.

The mental, social and physical age and the teenager's total situation ought to be considered when deciding the appropriate treatment. Interventions in oligomenorrhea or amenorrhea involve several different areas. Regulation of the menstrual disorder as well as treatment of the associated factors must be considered. Those with a clear PCOS diagnose should be informed about the condition and the significance of body weight for the clinical manifestations.

Girls with serious eating disorders should be referred to a specialist clinic. If this is not acceptable to the girl, treatment by an experienced psychologist in cooperation with a dietician and a gynecologist could be an alternative. If major depression or other severe psychiatric symptoms are present, the girl needs to be referred for consultation in a psychiatric clinic.

It is often important to involve the parents in the planning and implementation of the treatment. The evaluation of problems in the family or other social problems requires the competence of a social and guidance counselor at the clinic or the social services in the girl's living area.

When treating adolescents with menstrual disorders, it is essential with continued follow-up of interventions, menstrual status and weight development. Rapid weight loss and continued menstrual problems will be noticed at follow-up. If the girl agrees, collaboration with the school health clinic and the school nurse is a possibility.

Girls with low bone mass need information about ways to promote bone health. Nutritional recommendations must be adjusted to their specific activity level. Supplementing calcium and vitamin D3 may be beneficial. Girls with extreme low values of BMD should be referred to a specialist in bone health.

Youth clinics play an important role in adolescent health in Sweden today, with profound knowledge about teenagers that is lacking in other health care units. The results presented here suggest that further efforts for youth clinics, with a widening of their competence to provide multi-professional teamwork, could be a way to improve reproductive health in adolescents.

4.6 LIMITATIONS

The Adolescent study (I) was a restrospective study. Although the same gynecologist evaluated all the patients, the girls were not followed in a complete and totally structured manner. Furthermore, it would have been desirable to compare the results with a control group of girls with regular menstruations. There were no difference in the evaluation of the girls with secondary amenorrhea and oligomenorrhea except for evaluating those with secondary amenorrhea with imaging of the pituitary or a gestagen test.

In the follow-up studies in adulthood (II, III), there was a high drop-out rate from the initial investigation, since many of the women could not be reached by mail six years later. Among those who accepted the invitation, there were no difference in clinical variables between the groups of women with secondary amenorrhea and those with oligomenorrhea in adolescence. The present results are estimated to be representative of the initial population. Another limitation was that hormone analyses were only performed in women with persistent menstrual dysfunction.

Bone mineral density was only measured in adulthood (III) and could not be compared with a measurement in adolescence. Thus, the change in BMD values could not be estimated. However, BMD values could be evaluated as T- and Z-scores. Furthermore, there was no control group.

One limitation in the Life style and sexuality study (IV) was difficulties to estimate external validity by comparisons with other surveys due to differences in the age groups studied and in the formulation of the questionnaire. The high response rate served as an indication of high sample validity. Another limitation concerned reliability, which could not be assessed by test – retest because of the anonymity of the responses. Instead, the scale reliability was tested by Chronbach's alfa, which showed somewhat unsatisfactory results for some of the items. Possible explanations for this could be an incorrect choice of sub-items, difficulty in understanding or responding to sub-items, and responses that were not serious.

5 GENERAL CONCLUSIONS

Hypothalamic amenorrhea and hyperandrogenism were found to be the specific endocrine mechanisms of menstrual disorders in adolescent girls. Hypothalamic inhibition of the HPG-axis was related to anorectic eating behavior and dominated in the amenorrheic group, whereas a hyperandrogenic hormonal pattern, PCOS and eating disorders of bulimic type were frequent in the oligomenorrheic group. It is important to evaluate endocrine patterns, as well as eating behavior in adolescents with menstrual disturbances to ensure adequate medical care and treatment.

Menstrual dysfunction and eating disorders were still frequent at follow-up six years later. In these adult women, PCOS was found to be the main endocrine mechanism behind persistent menstrual disorders. Present eating disorder (EDNOS) had no significant influence on menstrual function. Recovery from anorectic eating disorders, which was most common in those with secondary amenorrhea in adolescence, was a strong predictor of resumption of menses. The findings call for a continued follow-up of women diagnosed with menstrual disturbance in adolescence.

A high frequency of osteopenia/osteoporosis in the femoral neck or lumbar spine was found in adult women who had had menstrual disorders in adolescence. Previous anorectic behavior was the strongest predictor of low BMD, whereas menstrual disorders as such had no negative influence on bone mass. The results emphasize the need for diagnosing and treating an underlying eating disorder, especially of the anorectic type in young women with menstrual dysfunction in order to prevent osteopenia.

The survey revealed that girls were less satisfied with life, their bodies and sexuality than boys. Weight control behavior was notably common among teenage girls and may indicate an eating disorder. The overall results also show that a proportion of the girls and boys constitute a risk group for adverse reproductive health due to alcohol abuse, non-use of contraception and family problems. Gender differences should be taken into consideration in the provision of adequate care and treatment for young people.

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