Aspects on Chronic Stress and Glucose Metabolism in Women with Recurrent Vulvovaginal Candidiasis and in Women with Localized Provoked Vulvodynia

Sophia Ehrström
ASPECTS ON CHRONIC STRESS AND GLUCOSE METABOLISM IN WOMEN WITH RECURRENT VULVOVAGINAL CANDIDIASIS AND IN WOMEN WITH LOCALIZED PROVOKED VULVODYNIA

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Abstract

Objective: To evaluate the degree of stress in women with recurrent vulvovaginal candidiasis (RVVC) and in women with localized provoked vulvodynia (LPV) (former vulvar vestibulitis syndrome) compared with controls. To measure the change of glucose in plasma and in vaginal secretions during oral glucose tolerance testing (OGTT) in women with RVVC, and in healthy control subjects.

Material and Methods: Altogether 147 women participated in the studies. A careful vulvovaginal examination was performed and a health questionnaire was completed. In some women, saliva for analysis of cortisol was collected 4 times in the morning and once in the evening. The analysis was performed with a time-resolved fluorescence immunoassay. A questionnaire about perceived stress at work and in private life was completed. Another part of the women underwent OGTT. Vaginal secretion from the proximal part of the vagina was collected on filter papers. Glucose in plasma and in vaginal secretions was measured at fasting and after two hours. In a subgroup of women with RVVC and control subjects, glucose in vaginal secretions and in plasma was collected every half-hour during OGTT. All samples were analysed with the hexokinase method.

Results: Blunted morning rise cortisol was registered more often in women with RVVC (p<0.002). Mean levels of salivary cortisol were lower the first 45 minutes after awakening in women with RVVC, compared with controls. Blunted morning rise cortisol was registered more often in women with LPV (p<0.05), compared with controls. Both women with RVVC, and women with LPV reported signs of burnout (p<0.001 and p<0.005), emotional symptoms of stress (p<0.005 and p<0.05), bodily symptoms of stress (p<0.05 and 0.005), and presented type D-personality (p<0.05). Moreover, women with RVVC perceived more worrying factors at work (p<0.05), and an impaired balance between work and leisure time (p=0.01).

More women with RVVC than controls reported a history of condyloma (p<0.001), and bacterial vaginosis (p<0.001). No differences were seen between women with RVVC and controls regarding SHBG, DHEA-s, testosterone or HbA1C.

In healthy women, the median level of glucose in vaginal secretion was 5.2 mmol/L before and 5.7 mmol/L after OGTT, and plasma glucose was 5.0 mmol/L before and 5.8 mmol/L after OGTT. No significant difference was seen regarding change of glucose level in vaginal secretions, and plasma glucose after, compared with before OGTT. Neither was there any difference between women with RVVC and controls regarding vaginal and plasma glucose levels every half hour during OGTT. Hemoglobin A1C and body mass index did not differ between the groups. In oral contraceptive users glucose in plasma 60 minutes after intake of 75 g of glucose (p<0.005) was higher than in women not using oral contraceptives.

Conclusions: More women with RVVC and LPV than controls showed signs of chronic stress. The evaluation was performed with two different techniques.

There were no differences between women with RVVC and control subjects regarding change in glucose level in vaginal secretions or in plasma, during OGTT. Vaginal glucose levels did not rise in oral contraceptive users during OGTT, in spite of higher plasma glucose levels 60 minutes after intake of 75 g of glucose. There were no differences in plasma or vaginal glucose levels before and after OGTT.

Key words: Recurrent vulvovaginal candidiasis, localized provoked vulvodynia, vulvar vestibulitis syndrome, Hypothalamic Pituitary Adrenal-axis, salivary cortisol, chronic stress, morning awakening cortisol, oral glucose tolerance testing, glucose in vaginal secretions, plasma glucose, body mass index

LIST OF PUBLICATIONS

This thesis is based on the following papers, which will be referred to in the text by their roman numerals.

I. Signs of Chronic Stress in Women with Recurrent Candida Vulvovaginitis
Sophia M Ehrström, MD, Dan Kornfeld, MD, PhD, Jessica Thuresson, MD, Eva Rylander, MD, PhD

II. Perceived Stress in Women with Recurrent Vulvovaginal Candidiasis
Sophia M Ehrström, MD, Dan Kornfeld, MD, PhD, Eva Rylander, MD, PhD
*Journal of Psychosomatic Obstetrics and Gynecology*, in press

III. Glucose in Vaginal Secretions before and after Oral Glucose Tolerance Testing in Women with and Without Recurrent Vulvovaginal Candidiasis
Sophia M Ehrström, MD, Anna Yu, MD, PhD, Eva Rylander, MD, PhD

IV. Influence of Oral Glucose Tolerance Testing on Glucose in Plasma and in Vaginal Secretion in Women with and without Recurrent Vulvovaginal Candidiasis with Reference to Oral Contraceptive Use
Sophia M Ehrström, MD, Anna Yu, MD, PhD, Eva Rylander, MD, PhD
*submitted*

V. Chronic Stress in Women with Localised Provoked Vulvodynia
Sophia M Ehrström, MD, Dan Kornfeld, MD, PhD, Eva Rylander, MD, PhD, Nina Bohn-Starke, MD, PhD
*submitted*

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"..barn måste jag ha, ty utan barnskrik kan jag inte arbeta.." (Strindberg.A)
Figure 1. Recurrent vulvovaginal candidiasis

Figure 2. Localized provoked vulvodynia (former vulvar vestibulitis syndrome): Erythema localized around the Bartholini gland openings and in fossa navicularis
Thomas Annersten and Rolf Andersson for instant and skilful service at the Photographic Unit at Danderyd Hospital.

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INTRODUCTION

Anatomy

The vulva forms the external part of the female genital organs. It includes the mons pubis, the clitoris, the meatus urethrae, the labia majora, the labia minora, the vestibule, the hymen, the vaginal orifice (Figure 3). The vagina is a dynamic fibromuscular canal connecting the vulva and the internal genital tract, i.e. the cervix of the uterus, the uterus, tubae and ovaries. The anterior wall neighbours the urethra and the bladder and the posterior wall lines the rectum. The proximal part of the vagina forms a cuff around the protruding cervix of the uterus, forming the anterior, lateral and posterior fornices. The vaginal mucosa consists of numerous folds, built up by longitudinal columns and transverse rugae and is extendible under the influence of steroid hormones, during sexual arousal and childbirth.

![Anatomy of the vulva](image)

**Figure 3: Anatomy of the vulva**

The vulva is supplied by the internal pudendal artery. The vestibulum vulva is supplied by the vaginal artery. The vagina is supplied by branches from the internal iliac artery. The upper part
of the vagina is supplied by the vaginal artery, the mid-vagina is supplied by the middle rectal artery, and the inferior internal and external pudendal arteries supply the lower part [1].

The vulva and the vestibule are lubricated by the vestibular glands. The orifice of the largest ones (the Bartholini glands) are located on each side in the groove between the hymen and the posterointerior part of the labia minora. The Skene glands drain on either side of the urethral meatus (Figure 3).

The vestibulum vulvae is innervated by the pudendal nerve, which originates from the sacral nerve roots S2-S4. This nerve contains somatic motor efferents and sensory afferents, and autonomic nerve fibres from the inferior hypogastric plexus and caudal sympathetic chain ganglia [2]. The vulvar vestibular mucosa is by definition visceral tissue, however it is considered to have a non-visceral innervation [3]. Thus, sensations of touch and pain are similar to sensations evoked in the skin. The external genitalia are innervated with both myelinated and unmyelinated nerves [4]. The labia minora are rich in most nerve endings involved in the perception of touch, pressure and pain [4]. Intraepithelial free nerve endings are present in the distal part of the vagina, the vestibule and the hymen [5]. The innervation of the remaining vagina is controversial.

**Histology**

In menstruating women, the vaginal mucosa forms numerous transverse folds and the mucosal lining consists of a non-cornified stratified squamous epithelium with about 20 cell layers. The intermediate and superficial cells are rich in glycogen. Due to the effect of estrogen, glycogen accumulates during the follicular phase of the menstrual cycle, and reaches its maximum before ovulation. The parabasal cells and the basal cells are active proliferative areas with stem cell properties. The basal layer constitutes the border with the underlying layer, the lamina propria, consisting of connective tissue rich in elastic fibers, and numerous small blood vessels. The muscularis consists predominantly of smooth muscle fibers. Interstitial connective tissue is rich in strong fi brocollagenous elastic fibers. The adventitia below the mucosa contains numerous blood vessels, lymph vessels and nerve bundles [1].
Physiology of the vagina

During the influence of estrogen, the endocervical glands secrete clear, watery mucus, which is abundant at the time of ovulation, facilitating sperm transport. Due to the progesterone production in the luteal phase, the mucus becomes thick and opaque. The vaginal secretion consists of a transudate through the vaginal epithelium, containing proteins, polysaccharides, free fatty acids, immunological factors, iron, lactoferrin, mucin, and proteolytic enzymes. The acid environment of the vagina (normal pH is approximately 4) is maintained by production of lactic acid and hydrogen peroxide from lactobacilli [1].

Basic immunology of the vagina

Lactobacilli inhibit colonization of potentially harmful microbes by producing hydrogen peroxide and lactic acid through fermentation of glycogen from the desquamated cells in the vaginal mucosa. Actors of the innate immunity are the first-line defense during the initial exposure to organisms (within hours), consisting of phagocytes (neutrophils, monocytes and macrophages), i.e. cells releasing inflammatory mediators (basophiles, mast cells and eosinophiles) and natural killer cells (NK-cells). The humoral components of the innate immunity include the complement system, acute-phase proteins and antimicrobial proteins and peptides, such as lysozyme and defensins. The adaptive immune system is activated by the innate immunity, and generates a memory against the infection within days [6-11]. The cell-mediated immunity is active in the defence against intracellular pathogens, bacterial agents of the STDs and virus, through cytotoxic T-cells [12] [13].

Bacteriology of the vagina

The normal bacterial flora of the vagina is complex and not fully understood. Döderlein recognised lactobacilli as the dominating commensal of the vaginal flora. The most common lactobacilli in the vaginal ecosystem are l.crispatus, l.jensenii, and l.gasseri [14]. In the 1930s, anaerobic bacteria were discovered in the vaginal fluid, and it was later shown that anaerobic bacteria such as peptococci, bacteroides and peptostreptococci dominate aerobic bacteria in the vagina [15]. The most common aerobic bacteria are staphylococci, streptococci and E.coli. Candida albicans is common in the vaginal secretion [14].
Fungi

Fungi are eukaryotic organisms, and possess a nucleus enclosed by a nuclear membrane. Fungi lead a heterotrophic existence as saprobes, symbionts, commensals or parasites. They exist in two basic morphologic forms, yeast and hyphae. Yeast is unicellular and reproduces asexually by blastoconidia formation. The hyphae are branching, threadlike tubular filaments.

![Figure 4. Pseudohyphae and spores Candida albicans](image)

The most common yeast *Candida albicans* is a commensal in the oral cavity, in the gastrointestinal tract, and in the vagina. In the transition from colonization to infection, *Candida albicans* forms germ tubes, spores, pseudohyphae and hyphae [16] (Figure 4). Hyphae may penetrate the epithelium (Figure 5). The formation of germ tubes is inhibited by *lactobacilli*, by way of a coating effect [17]. In vitro, the formation of germ tubes is stimulated by glucose [14]. Candida species are not associated with an elevated pH-level in the vagina and they may coexist with *lactobacilli* in vaginal fluids, in contrast to bacterial vaginosis. Example of yeast species that may grow in vaginal secretions are *C.albicans* (90%), *C.glabrata* (4%), *C.parapsilosis*, *C.tropicalis*, *C.krusei* and *Saccaromyces cereviciae*. 
Recurrent Vulvovaginal Candidiasis

Introduction

Vulvovaginal candidiasis is a common disorder in women of fertile age. Approximately 75% of all women will experience an episode of vulvovaginal candida infection at least once in their lifetime [18], and as much as 5 to 8% of women of reproductive age suffer from repetitive episodes of vulvovaginal candidiasis, which may have a severe impact on the relationship and the quality of life of the woman and her partner [18, 19]. Recurrent vulvovaginal candidiasis (RVVC), is defined as having four or more episodes of vulvovaginal candida in a 12 month period [18-21].

Symptoms

The most common symptoms are itching, soreness, dryness, and perineal fissures that often cause dyspareunia. Erythema, dysuria or vulvovaginal edema may be present. Women with acute vulvovaginal candidiasis may have a typical thick whitish discharge, while women with RVVC most often have a dry mucosa with little or no discharge at all (Figure 1). Sexual intercourse may be uncomfortable and often painful. Many women with RVVC report a burning sensation and soreness of the vulva that may persist for hours or days after intercourse. Some women may develop localized provoked vulvodynia (former vulvar vestibulitis
syndrome). To avoid this refractory disorder, it is important to treat the vulvovaginal candida infections appropriately.

Prevalence

The first suggestion that vaginitis may be due to yeast was made in 1849 by JS Wilkinsson [22]. In 1916, it was shown that yeast could be asymptomatically present in the vagina [23]. The existence of asymptomatic carriers of candida has given rise to great variation in the reported prevalence of vaginal candidiasis. In the 1960s, the prevalence of asymptomatic colonization of Candida albicans in Britain was reported to be approximately 16 % in non-pregnant women, and 25 % in pregnant women [24]. Recurrent vulvovaginal candidiasis has become common the last two decades. In a recent longitudinal cohort study including an STD-clinic, and a student health center in the US, 30 % of the women were shown to be asymptomatic carriers of Candida albicans [25]. At an adolescent health center in Sweden in 2002, the prevalence of candida was 42 %, while 15 % of these women were asymptomatic[26]. Vulvovaginal candidiasis is rarely seen before menarche or in postmenopausal women that are not using hormonal replacement therapy. During the last 15 years, the use of topical antifungals has increased with 50 % both in the USA and in Sweden, possibly due to the fact that they are available over the counter (OTC) [27]. Ninety-three percent of all antifungal treatment in Sweden are sold as OTC products [27]. Women with recurrent vulvovaginal candidiasis represent 26% of all the patients who are referred from other gynecologists to the Vulvar unit at Danderyd hospital, Stockholm, Sweden.

Pathogenesis and background factors

The pathogenesis of RVVC is poorly understood, despite decades of research. Candida is an opportunistic organism in the human body. It may give rise to symptomatic vulvovaginal infections under certain circumstances. Use of antibiotics has been suggested as a risk factor for both acute [18] and recurrent vulvovaginal candidiasis [28, 29]. Other causes that have been discussed, such as poorly regulated diabetes [30], pregnancy [19, 31], long time treatment with immunosuppressive agents as corticosteroids or chemostatics [19, 31] only partly explain the pathogenesis of RVVC [19]. There are many possible etiological factors, but candidiasis is usually described as idiopathic, i.e. with no known predisposing factors present in the woman. It is possible that the susceptibility to RVVC also is dependent on the host, and not only on external factors.
Genetic and immunological factors
Genetic factors that may increase the receptiveness to chronic vulvovaginal candidiasis have been discussed recently. Babula and coworkers registered that women with RVVC have got reduced vaginal levels of anticandidal factors in IL-4* homozygotes, which may increase susceptibility to RVVC [32]. Moreover, strain on the host may impair the function of the immunologic barrier of the vaginal epithelium and thus initiate vulvovaginal candidiasis. Candida-specific cell-mediated immunity, acquired by exposure to candida as a commensal early in life has been considered the predominant host defense mechanism against mucosal candida infections [33-35]. Candida-specific antibodies are not considered to play a role in protection against infection [35, 36]. Allergy has been discussed in the 1990s without conclusive findings [37, 38]. Associations of RVVC with rhinitis [39], and atopy [40] [41, 42] have been observed. Recent data suggest that symptomatic VVC is associated with an aggressive response by polymorphonuclear neutrophils i.e. protection against and promotion of candida vulvovaginitis through the innate part of the immune system [36].

Oral contraceptives
Some studies have shown that women using oral contraceptive more often get recurrences of vulvovaginal candidiasis compared with non-users, while oral contraceptives (OC) per se do not seem to affect the occurrence of sporadic VVC [28, 43]. Historically, the data are not conclusive [44, 45]. Frequent sexual intercourse in OC users may also contribute to the activation of Candida infection [28, 46]. Women using OC have been shown to have minor changes in plasma insulin and glucose tolerance, possibly an effect of progestogen [47]. There is no evidence of an increase in manifest diabetes mellitus due to the use of OC.

Sexual and hygiene habits
The role of sexual habits in the activation of candida has been evaluated. Frequent sexual intercourse [28, 31] [46] [48], several lifetime sexual partners [28, 31, 46], and oral sex [26] have been suspected to increase the risk for developing recurrent vulvovaginal candidiasis. The use of intrauterine device has also been discussed [28, 31]. However, no consistent results have been presented. Allergy to semen of the partner or products in the semen derived from what the partner may have ingested, has been reported in a few cases [49]. Hygiene habits that are associated with RVVC include repeated cleansing as well as shaving of the genital area, vaginal douching, and the use of soaps low in pH [31], which will cause drying
out of the vulvar mucosa and micro lesions in the skin barrier. The use of panty-liners is associated with the existence of RVVC [50] [26, 44]. It is however not clear if these hygiene habits are possible causes for developing RVVC, or consequences of the discomfort of having repeated vulvovaginal complaints.

**Glucose control**

Often, women with RVVC are advised to abstain from carbohydrates, as a prophylactic aim. It is known that insulin-dependent diabetics with poorly regulated glucose levels easily will develop vulvovaginal candidiasis. Yeast grows in vitro in a sugar-rich environment [51]. However, in the literature there exists very little scientific evidence for the beneficial effect of a carbohydrate low diet. Horowitz found elevated levels of glucose, arabinose, and ribose in the urine of subjects with recurrent and ongoing vulvovaginal candidiasis [52]. Donders et al. registered elevated plasma levels of glucose 60-90 minutes after intake of 75 g of glucose in women with RVVC.

**Diagnostic methods**

A careful clinical examination of the vulva and the vagina is necessary for an accurate diagnosis. Differential diagnoses such as bacterial vaginosis (co-existence is common), condyloma, herpes genitalis, or irritative dermatitis should be ruled out.

The most common method to diagnose vulvovaginal candidiasis is by a wet mount. However, hyphae are detectable in only 60 % of cases with ongoing vulvovaginal candidiasis (i.e. the sensitivity of the test) [53]. Culture from vaginal samples, placed on Sabouraud and CHROMagar®, is a more reliable method with a sensitivity of approximately 90 %. PCR-detection of Candida strains has promising sensitivity and is under development, but is not yet in clinical use. Vaginal yeast culture is recommended in recurrent cases, when there are no signs in wet mount, when information of species of yeast is needed and when antifungal resistance is suspected [54].
Treatment

Asymptomatic carriers of *Candida albicans* should not be treated.

Sporadic occurrence of vulvovaginal candida is treated with local clotrimazole or nystatin, or with a single dose of oral fluconazole or itraconazole.

Antimycotics are bacteriostatic. However, recurrences of vulvovaginal candidiasis may be suppressed with oral fluconazole [55]. Concentrations of fluconazole above the MIC-value (when 90% of all candidal growth is inhibited) is only achieved for 72 to 96 hours after oral administration [54, 55]. Thus, relapsing cases should be treated by oral fluconazole 150 mg twice a week or 50 mg daily, sometimes for 1-3 months. Free interval between infections may be prolonged after such a long-time suppression. This treatment is safe, effective (93 %) and have few side-effects [54, 55]. Ketoconazole may be used in severe cases for shorter treatment periods only, due to liver toxicity.

Oil regime is recommended, and excessive use of water and soap should be avoided due to the risk of drying out the tissues. Topical ointments other than oil, such as cortisone cream and local antimycotics should be avoided in women with RVVC due to the risk of developing local irritation and hypersensitivity reactions in the vulnerable vestibular mucosa.

Resistance against fluconazole for *Candida albicans* and *Candida parapsilosis* is rare in Sweden, but a few cases have been reported from the US [56]. Species other than *Candida albicans* (94%), such as *Candida glabrata* (4%), *Candida krusei*, and *Saccaromyces cerevisiae* are frequently resistant to fluconazole [56]. Local treatment with Clotrimazole 200 mg x1xXII or Boric acid capsules 600 mg x2xXIV are two alternative treatments in these cases.

Sexual partners often harbour identical strains of *Candida albicans* in the vagina, orally, and in semen. However, this fact has not been able to predict recurrences. Partner treatment has not proven to be efficient in preventing RVVC [41, 57, 58] and is thus not recommended [41].
Localized provoked vulvodynia (former vulvar vestibulitis syndrome)

**Definition**
Localized provoked vulvodynia (LPV), (former vulvar vestibulitis syndrome- a terminology that has been used for many years but was recently proposed to be changed to LPV) [59] is characterized by a hypersensitive mucosa around the Bartholini glands, fossa navicularis and the hymeneal ring, and duration of pain of at least six months. Localized erythema in the mentioned areas is generally present [60] (Figure 2). The pain is always provoked by intercourse and tampon use, and sometimes by tight clothing and cycling. The pain is described by most patients as burning or stinging. Sexual intercourse is very painful or impossible to perform.

**Incidence and prevalence**
The incidence of LPV is not known, but the number of patients has increased during the last 15 years, [61] [62, 63].
The prevalence of LPV in the general population is difficult to define, due to the fact that many studies are based on retrospective questionnaires and/or selected populations. Vulvar pain may be due to unprovoked vulvodynia or to recurrent vulvovaginal candidiasis, bacterial vaginosis, condylomata, vulvar dermatoses, and other less frequent diagnoses. According to a population based study in the USA, 16% of women in the Boston area suffered from vulvar pain [64]. However, most participating women were not clinically examined. In Sweden, about 13% of women aged 20-29 years who attended an outpatient clinic reported a history of long lasting superficial pain at intercourse[65].

**Pathogenesis and background factors**
The pathogenesis of LPV is multifactorial, and not fully understood. Many etiological factors have been discussed. In a recent web-based survey, women with LPV listed yeast infections and stress as the two factors that contributed most to the development of vulvar pain. Other mentioned contributing factors were antibiotics, bacterial infection, birth control pills, chemical irritation trauma to area or cause unknown. Moreover, 55 % reported that stress aggravated their symptoms [66].
The women with LPV are usually young oral contraceptive users, and have generally not given birth [63]. In some women previous local treatment of the vulvar area due to candida and/or condyloma may have initiated the pain [67, 68]. Up to 75% of women with localized provoked vulvodynia have a history of recurrent candida vulvovaginitis [67]. Vaginismus or tension in the pelvic floor musculature is often present [69]. (Figure 6)

Figure 6. Factors that may be involved in the pathogenesis of localized provoked vulvodynia

Negative experiences related to emotional, psychological and sexual factors are possible contributing etiological factors in some women. The psychological morbidity in women with LPV has been investigated in several studies. Both the presence and the absence of psychopathology have been reported [70-73]. It is not clear whether this reflects the cause or effect of the pain [70]. In clinical practise some women with LPV seem to be depressed, and in self-reported questionnaires, women with LPV report higher rates of depression and anxiety than controls [74] [73, 75]. Women with LPV have been characterized as perfectionistic, cautious, careful, insecure and pessimistic [74, 76] [77].

Compared with controls, there is an increased peripheral innervation and sensitization of nociceptors in the vestibulum vulvae. These findings together with increased mucosal blood
flow indicate a present neurogenic inflammation of the vestibular mucosa in women with LPV [78]. Pain from other parts of the body is common in vulvodynia patients [65, 79], as well as generalized systemic hypersensitivity with lowered pain thresholds for various stimuli in extremities [80-82].

**Treatment**

Localized provoked vulvodynia is a complex disorder that is difficult to treat. The following options may be considered in the treatment:

1. Avoid self-prescribed local treatments and irritants
2. Interruption of oral contraceptives for at least 4-6 months
3. Topical lidocaine gel several times daily or during the night [83]
4. Prevention and/or treatment of secondary vaginismus, by training to relax of the pelvic floor muscles [84]
5. Amitryptiline 50-75 mg at night to inhibit the pain circle. Gabapentin up to 2700 mg daily may also be tried [84, 85]
6. Biofeedback training
7. Cognitive behavioural treatment [84]
8. Sexual counselling
9. Surgery [86]

The treatment should be individualized. At least 15% of women with LPV are relieved from pain through omitting of oral contraceptives. The use of topical lidocaine several times daily, in order to desensitize the superficial nerve endings of the vulvar vestibular mucosa, will be more effective the earlier the treatment is started [83]. A number of measures can be taken to prevent irritation, and medications such as amitryptiline and gabapentin may be used to elevate the pain threshold. Oral antimycotics may be used in case of a superimposed candida infection. At our vulvar clinic, we may include physical therapy and counsellors addressing stress and lifestyle in women with LPV. Many patients improve their coping skills and report reduction of stress symptoms after this counselling (unpublished data). Spontaneous remission may occur after years [87].
Stress

Introduction

Since the work of Selye in 1950, stress has been associated with the activation of the HPA-axis [88]. The fight-or-flight reaction, originally described by Walter Cannon in 1910, is activated when an organism is threatened by external factors, resulting in an activation of the sympathetic nervous system (SNS) [89]. This situation will produce an elevation of pulse and blood pressure, a redistribution of blood to the limbs, and mental arousal. The two major stress-systems in the body, the SNS and the Hypothalamus Pituitary Adrenal axis (HPA-axis) both interact with the hormonal system and the immune system on a central and a peripheral level [90] [91] [92].

Initially in stress research, physical stressors and acute stress reactions were studied. Since the 1970s psychosocial factors have been evaluated in addition. This has given a more complex picture to the associations between environmental and individual conditions. Lately, long term effects of stressors and their negative effect on homeostasis resulting from overload (allostatic load) have been investigated [93]. Chronically activated HPA-axis and sympathetic nervous system may cause disease [93]. Constantly ongoing stress impairs the immune system and may thus result in an individual more prone to infections [94].

The Hypothalamus Pituitary Adrenal axis

The HPA-axis is a feedback system. The activation of the HPA-axis by stressors results in release of Corticotrope Releasing Hormone (CRH) from the hypothalamus, ACTH from the pituitary gland and cortisol from the adrenal glands. Increasing levels of cortisol inhibit CRH-release from the brain, and attenuates the activity of the SNS [92] [95] [96]. CRH-release is stimulated by catecholamines and immune factors [95]. A stressor is defined as a factor that affects the homeostasis. A stressor can be physical, chemical, psychological or social [97]. Emotional stress and other mental stress are powerful stressors.

The HPA-axis has a characteristic diurnal pattern [98]. One hour before awakening, the level of cortisol peaks, and then rises substantially after awakening and somewhat after lunch, diminishes throughout the day, and reaches the lowest levels before bedtime (Figure 7).
Acute stress
In a situation of acute stress, the catecholamines epinephrine (E) and nor epinephrine (NE) are instantly secreted into the circulation. NE is responsible for blood pressure control, and is influenced by physical activity and body posture. E is influenced by mental and physical stress. Both E and NE stimulate the activity of the HPA-axis. Cortisol is released 20 minutes after the encounter with the stressor, which has effects on arousal and activation of the immune system. E and NE act in the periphery and do not pass the blood-brain barrier. Cortisol may pass the blood-brain barrier. Clinical and experimental studies have shown that the immune system is boosted after acute stress [99]. Mental stress is a strong activator of both epinephrine and cortisol [100].

Chronic stress
Repetitive confrontation with stressors will initially increase the response of the HPA-axis, but eventually attenuate the cortisol response (Figure 8), [101, 102]. Hypo responsiveness of the HPA-axis is typically represented by a blunted morning awakening (or “morning rise”) cortisol [103] [100, 104, 105].

Figure 7. Diurnal pattern of cortisol
In animals, the effect of the immune system is impaired in experimental situations of chronic stress[106-108]. In clinical studies it has been shown that chronic stress impairs the immune system, in particular the innate part [109-111]. Blunted morning rise cortisol has been registered in atopic and asthmatic children[97]. McEwen and Dhabar have investigated the negative effects of allostatic load, i.e. demands exceeding stress protecting mechanisms [93, 112]. This overload results in an imbalanced homeostasis, with susceptibility to disease as a consequence.

Hyper-responsiveness of the HPA-axis is well known to be associated with hypertension [113], abdominal obesity [114, 115], the metabolic syndrome [116], and osteoporosis [117]. Hypo-responsiveness of the HPA-axis has been associated with disorders such as chronic fatigue syndrome [118], low back pain [119, 120], chronic pelvic pain [121], fibromyalgia [120], post-traumatic stress disorder [110, 122], irritable bowel syndrome [123], burnout [124], anorexia nervosa [125], and atypical depression [126].

**Mental stress and burnout**

In modern society, mental stress is of much greater importance than physical stress as an activator of the stress systems. The fight-or-flight reaction is often chronically activated. This may produce an allostatic load in the body. Physical stress will cause a more prominent
increase in epinephrine in men than in women, but emotional stress will have the opposite effect [127].

Burnout has been of major importance in studies regarding the development of work related disease in recent years, especially in women. Burnout is characterized by feelings of exhaustion, mental tiredness, and depersonalization [128]. Sleep disturbances are often present [129]. Lack of coping in the individual may produce susceptibility to stress, and development of burnout [130]. Burnout is initiated by the situation at work in 2/3 and by private life in 1/3 [131].

Type A and Type D-personality have been discussed as important factors in the development of disease in individuals living or working in a stressful environment. A person with Type A personality is characterized by feelings of hostility, anger, impatience, anxiety and stress [132], while a person with Type D-personality is characterized by having a negative self-picture, depressive traits with feelings of hopelessness and helplessness [133]. Type A personality is overrepresented in patients with coronary heart disease [132], hypertension and the metabolic syndrome [134], and Type D personality has been linked to depression [135] and burnout [136].

Mental stress and depression

The effect of depression on the regulation of the HPA-axis has been evaluated. There is a hyper responsiveness of the HPA-axis in patients with major or atypical depression [137], while patients with minor depressive symptoms present a hypo responsiveness of the HPA-axis, expressed with blunted morning awakening cortisol [138]. Elevated serum and CSF levels of the proinflammatory cytokine IL-6 [139], deficient natural killer (NK)-cell activity and impairments in T-cell proliferation [140] have been seen in patients with major depression, depending on the severity of the depression.

Psychoneuroimmunology

The immune system can be divided in two parts. The innate part is active in the defense against mucosal infections and includes granulocytes, macrophages and natural killer (NK)-cells ref). NK-cells and leukocytes express receptors for neuropeptides [141], and other hormone-regulating interactions between the brain and the immune system. NK-cells and macrophages produce cytokines. Their key function is immune surveillance and regulation and communication with other parts of the immune system. Situations of acute stress increase the serum level of NK-cells and granulocytes [142, 143], while several studies have shown that
chronic psychological stress may induce lower levels of NK-cells, granulocytes and macrophages, as well as impaired function [144]. The acquired part of the immune system consists of B-cells and T-cells (lymphocytes). B-cells from the bone marrow produce and secrete antibodies into the blood stream in response to different antigens. T-cells are differentiated in the thymus and are sub grouped into T-helper cells, (active in initiation of the specific immune response by stimulating other immune cells), and T-cytotoxic cells, (kill virus infected cells and tumour cells). Cytokines are proteins that act as messengers between lymphocytes. A stressful situation will induce an immune activation in which the proinflammatory cytokines IL-1 α, IL-6 and TNF-α are released.

There is a complex interaction between the nervous system, the hormonal system and the immune system. Cytokines, hormones, neurotransmitters and neuropeptides are active in the communication between these systems. By acting on the CNS, production of cytokines in the brain and in the peripheral organs induces fever, behavioural changes, hormonal secretions and autonomic reactions [145] [146]. Moreover, there are adrenergic and cholinergic receptors on lymphocytes [147, 148], and synapses for T-lymphocytes and macrophages at the nerve endings, indicating a direct communication between the nervous system and the immune system [149]. Peripheral nerves secrete peptides such as calcitonin gene-related peptide (CGRP) and substance P, which have a powerful effect on immune cells [150].

Psychoneuroimmunology and disease
Glucocorticoids are the most potent anti-inflammatory hormones in the body. They act on the immune system both by suppressing and stimulating pro-and anti-inflammatory mediators. They inhibit anti-inflammatory responses and suppress the production and release of pro-inflammatory cytokines such as TNF-α, IL-1 and IL-6 [151]. Studies have shown that rats that have a weak HPA-function with low levels of cortisone are much more susceptible to infections, and to a variety of autoimmune and inflammatory conditions [108, 152]. Cohen and coworkers have suggested that such chronic stress as social isolation and unemployment, may increase the susceptibility to common colds [153, 154]. In autoimmune diseases as multiple sclerosis, rheumatoid arthritis, type 1 diabetes cortisol levels are often low, and stress has been shown to worsen these conditions [155]. Hypocortisolism is also present in fibromyalgia [120], chronic fatigue syndrome [118], post traumatic stress syndrome (PTSD), atypical depression [126], arthritis, asthma and atopic dermatitis [156] [97]. Blunted cortisol responses have been registered in women with chronic pelvic pain with a history of sexual and physical abuse [121].
Chronic stress and perception of pain

Pain is a normal protecting mechanism to avoid danger and trauma. Women with LPV have an increased peripheral innervation of the vestibular mucosa, compared to healthy women, indicating increased pain perception [78]. Vaginismus or tension in the pelvic floor musculature is often present in these patients as a mechanism of protection from repetitive pain traumata [69]. It has been shown, that mental stress increases the perception of pain in work related upper extremities disorders and low back pain [157]. Chronic stress will increase pain perception in the locus coeruleus area in the brain [156].

A possible pathway in the development of localized provoked vulvodynia might be that chronic mental stress will enhance the perception of pain, and a vicious circle may be established.

Management of chronic stress

The management of chronic stress is a complicated task. Long time treatment with a cognitive behavioral approach, medication and physical therapy is needed. A multidisciplinary approach is necessary.
Glucose metabolism

Since poorly controlled diabetes mellitus is a known risk factor for developing recurrent vulvovaginal candida infections [158, 159], there is a belief that dietary factors like excessive intake of carbohydrates or sweets might increase the risk of recurrent vulvovaginal candidiasis. However, there are few scientific reports concerning this topic. According to one study, women with recurrent vulvovaginal candidiasis have elevated urinary secretion of glucose during ongoing infections [52]. Another study suggested a slightly impaired plasma glucose tolerance in women with recurrent vulvovaginal candidiasis [158]. However, levels of glucose in vaginal secretions have not previously been measured.

In case of severe infections such as sepsis, bacterial infections may have an influence on the glucose metabolism in healthy individuals. The glucose level will initially rise, followed by a short period of hypoglycaemia, and eventually return to normal levels [159]. In diabetic subjects, the control of glucose in plasma is more difficult, due to insulin resistance. Diabetics are more prone to higher morbidity and mortality secondary to a severe bacterial infection. This fact is possibly due to immunologic changes, in particular an impaired innate response of the polymorphonuclear leukocytes [160]. The B- and T-cell function in diabetic subjects with well-controlled diabetes is normal, however [161]. A high level of HbA1C has been associated with vulvovaginal colonization of candida in diabetic subjects [162]. In another study, it was observed that diabetic women who were orally colonized with candida had higher oral glucose levels than diabetics and healthy control women without oral candida [163]. Although the occurrence of vulvovaginal candidiasis (VVC) in diabetic subjects has been studied [164, 165], previous measurements of glucose in vaginal secretions have not been made. This thesis further investigates the levels of glucose in plasma and in vaginal secretions during oral glucose tolerance testing in women with RVVC.
AIMS OF THE STUDY

Recurrent vulvovaginal candidiasis and localized provoked vulvodynia have become common in the last two decades. Approximately 10-15% of all fertile women suffer from repetitive vulvar symptoms such as itch, dryness, soreness or dyspareunia, causing severe impact on relationships and quality of life. Possible etiological factors in the development of RVVC and LPV have been discussed in numerous studies, without conclusive explanatory findings. The pathogenesis of these two linked vulvovaginal disorders remains largely unclear. The main purposes of these studies were to investigate aspects of lifestyle, such as chronic mental stress and intake of glucose, in relation to recurrent vulvovaginal candidiasis and localized provoked vulvodynia, with the aim to recommend preventive strategies.

This thesis is based on the following hypotheses:

1) There is an association between chronic stress and the tendency to get recurrent vulvovaginal candida infections
2) There is an association between localized provoked vulvodynia (former vulvar vestibulitis syndrome) and chronic stress
3) The vaginal levels of glucose will differ in women with RVVC and healthy controls before, during and after glucose load
4) The levels of glucose in plasma during oral glucose tolerance testing will reveal a tendency of insulin resistance in women with RVVC

Specific aims of studies I-V

To analyse morning awakening cortisol in women with RVVC.
To register the degree of perceived chronic stress and study lifestyle in women with RVVC.
To analyse morning awakening cortisol and register the degree of perceived stress and lifestyle factors in women with LPV.
To measure levels of glucose in vaginal secretions and in plasma before, during and after oral glucose tolerance testing in women with recurrent vulvovaginal candidiasis.
PARTICIPANTS

Ethics
All studies were approved by the local ethics committee of the Karolinska Hospital. The participants gave their written informed consent.

Subjects
Women with recurrent vulvovaginal candidiasis
Altogether fifty-seven women fulfilling the criteria for RVVC (at least 4 symptomatic infections with vulvovaginal infections in the last 12 months) were included in the studies. The patients were recruited from the Vulvar Unit at Danderyd hospital, Stockholm, Sweden, to where they had been referred from other gynecologists due to recurrent infections with culture positive vulvovaginal candidiasis. The participants had previously been repeatedly treated with various local and oral antimycotics. At the initial visit, a careful clinical examination was performed, and a wet mount and fungal culture were obtained from vaginal secretions. The women with RVVC were otherwise healthy, with no ongoing treatment for systemic disease. Two women with RVVC had successfully been operated for vulvar vestibulitis several years previously. Exclusion criteria were: pregnancy, any sexually transmitted disease, ongoing bacterial vaginosis, vulvar dermatosis, severe medical, psychiatric or psychological disorders and history of sexual abuse. The women did not fulfil the criteria for localized provoked vulvodynia. Thirty women participated in more than one study (Figure 9).

Women with localized provoked vulvodynia
Forty-one women with localized provoked vulvodynia participated in the study (Figure 9). The patients were regularly attending the Vulvar Unit at the Division of Obstetrics and Gynecology, Danderyd Hospital, Stockholm, Sweden. Inclusion criteria were pronounced pain during intercourse attempts, erythema and severe vulvar pain to light pressure from a cotton wool swab over the area around the Bartholini gland openings, and/or fossa Navicularis, and/or along the hymeneal groove, most often in the posterior fourchette. The duration of symptoms was at least six months and the women were 18 years or older. Exclusion criteria were: pregnancy, ongoing vulvovaginal candidiasis or bacterial vaginosis, any sexually transmitted disease, vulvar dermatosis, severe medical, psychiatric or psychological disorders and history of sexual abuse.
**Control women**

The 49 control subjects were healthy medical students and other healthy women, recruited by advertising in a local newspaper. Exclusion criteria were: pregnancy, dyspareunia, any vulvar pain, ongoing vulvovaginal candidiasis or bacterial vaginosis, any sexually transmitted disease, vulvar dermatosis, severe medical, psychiatric or psychological disorders and history of sexual abuse. Twenty-eight women participated in more than one study (Figure 9).

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**Figure 9. Participants in the studies**
METHODS

Self-reported health questionnaire

*Paper I-V*

Fifty-seven women with RVVC, 32 women with LPV and 49 healthy control women completed a self-reported questionnaire covering past and present gynecological complaints, dermatological problems, other bodily pain, sexuality, and lifestyle factors (Figure 9).

Morning awakening salivary cortisol

*The method*

In several investigations it has been demonstrated that the free cortisol response to awakening can serve as an index of adrenocortical activity. Salivary cortisol is regarded as a golden standard method for analyses of cortisol in stress research [166]. The salivary levels of cortisol are in a steady-state relation to the serum levels [166]. When analyzed repeatedly, the results are reproducible [167]. Neither age, nor the use of oral contraceptives, smoking, time of awakening or the use of an alarm clock have a considerable impact on free cortisol levels after awakening [167]. The sampling procedure is simple and non-invasive for the test person and is thus not a stressor in itself. Since intake of food or drinks may have an influence on the quality of the salivary cortisol samples, the test person must be fasting. There are gender-related differences in the morning awakening response. The amount of saliva flow that is produced does not affect the analysis [167]. At awakening, the salivary cortisol level is approximately 10 mmol/l, and within the first 30 minutes it will increase with 50%, normally (Figure 10). Morning awakening
cortisol is a sensitive method to detect hyporesponsiveness of the Hypothalamus-Pituitary-Adrenal axis (HPA-axis), as a sign of allostatic load and chronic stress.

**Paper I and V**

Thirty-five women with RVVC, 41 women with LPV, and 35 healthy control women of the same age collected samples for analysis of salivary cortisol (Figure 9). By chewing a device called Salivette (a cotton wool swab) for 40 seconds, salivary flow was stimulated and collected at awakening, after 15, 30 and 45 minutes, and before bedtime. The women were instructed to store the Salivettes in the freezer until bringing them to the hospital. The women were not allowed to drink, eat, brush their teeth, smoke or use snuff after bedtime, the night before the morning saliva was collected. The saliva-saturated devices were placed in test tubes and sent for analysis to the laboratory at the Institute of Physiological Psychology at the Heinrich Heine Universität in Dusseldorf, Germany. Salivary cortisol was analyzed with a time-resolved fluorescence immunoassay with an intra- and interassay variability of 5% and 8% respectively [168].

**Self-reported internet-based stress questionnaire Docco®**

**Paper II and V**

Thirty-three women with RVVC, 33 women with LPV, and 28 healthy controls completed an internet-based coded questionnaire Docco®, concerning self-reported chronic stress (Figure 9). Docco® has been developed and validated at the Karolinska Institutet, and is currently used at the Stress care unit at the Institute of Psychosocial Medicine at Karolinska Institutet in Stockholm, Sweden. There are not many validated questionnaires in use that analyze chronic stress. The Docco® questionnaire is based on experience from the Swedish society. Docco® consists of about 200 questions including perceived stress at work and in private life, emotional and bodily symptoms of stress, personality, and lifestyle. Each question has a four-graded scale (Appendix). A synthesis of the answers of the questions was constructed by a computerized program, developed by experienced psychologists and cognitive behavioral therapists. The results are presented as mean values, range 1-4, for each topic.
**Analyses of hormonal levels in plasma**

*Paper I*

Serum samples for analysis of hormones were obtained on days 5 to 11 of the menstrual cycle from 35 women with RVVC and 35 control women for analysis of S-TSH, S-cholesterol, S-albumin, S-DHEA-s, S-SHBG, S-testosterone, S-bioactive testosterone, S-estradiol and S-HbA1C (Figure 9). It has been shown that chronic stress may cause alterations in the levels of these hormones [169, 170].

**Analyses of glucose in vaginal secretions and in plasma**

*Paper III and IV*

Oral glucose tolerance testing according to the standard protocol in Sweden was performed in 44 women with RVVC, and in 38 controls (Figure 9). Glucose was measured in vaginal secretions and in plasma in the morning at fasting and two hours after intake of 75 g of glucose in all the participants. Vaginal and plasma samples were also collected after 30, 60, and 90 minutes in nine women with RVVC and in 11 controls (Figure 9). The hexokinase method was used to determine glucose. Vaginal fluid was collected on a sterile, weighed strip of filter paper once at fasting, and once two hours after intake of 75 g of glucose. The filter paper was inserted in the posterior fornix and kept there for three minutes. It was then placed in a weighed sample tube containing 20 mg sodium fluoride and 143 IU Na₂-EDTA (BD Vacutainer Systems, Plymouth, UK) to which 500 μl 0.9% NaCl had been added. The tube containing the strip of filter paper soaked in vaginal fluid was reweighed in order to determine the weight of the sample obtained. After mixing by tube inversions (10 min) and centrifugation (10 min, 3000 g) the supernatant was collected. The hexokinase method (Gluco-quant®, Modular Analytics, Roche Diagnostics, Mannheim, Germany) was used for analysis of glucose. The obtained results were adjusted according to sample weight and dilution. The precision of this procedure was evaluated as follows: Twenty-two pieces of filter paper were soaked in plasma and inserted in the 22 sample tubes as described above. The concentration of glucose was determined in the supernatants and adjusted for dilution. The coefficient of variation from 22 measurements was less than 3% for glucose (range 5.06-5.83 mmol/l). Direct measurements of glucose (5.22 and 5.18 mmol/l) in the same plasma sample gave results within the respective ranges.
STATISTICAL ANALYSES

Chi-square, odds ratio and Mann Whitney U-test were used to assess differences in the reported history of infections, gastrointestinal symptoms, allergies and other symptoms between patients and controls.

Regression analysis was used to calculate the slope of the morning rise in cortisol for all the participants of the studies regarding morning awakening cortisol. The model based mean relative values of the fitted regression lines at 45 minutes were used: For each individual, the slope and intercept of cortisol on time were calculated. Taking the ratio between the 45-min value and the intercept value formed the relative change in fitted cortisol values in women with recurrent vulvovaginal candidiasis. A comparison of this ratio between patients and controls was performed with ANOVA test. In women with LPV linear regression analysis was used to calculate the slope of the morning rise in salivary cortisol (the k-value). A comparison between the k-values (normal distribution) in patients and controls was performed with Students T-test. A comparison of the results of the stress questionnaire Docco® was performed with Students T-test and one-way ANOVA test (normal distribution).

The Mann Whitney test was employed to compare levels of steroid hormones between women with RVVC and controls.

The choice of statistical methods when comparing values before and after OGTT was independent tests between groups (Mann Whitney U-test and T-test) in the univariate tests and Analysis of variance in the multivariate tests. ANOVA within repeated measurements analysis of variance were used to analyze the change in repeated glucose values in vaginal secretions and in plasma during oral glucose intake. Analyses were performed in SPSS®, version 14.0, and the statistical software program STATISTICA®.
RESULTS

Demographical data and body mass index are presented in Table I. The most common symptoms in women with RVVC were itch, soreness, dryness and dyspareunia (p<0.0001). The most common complaint among women with localized provoked vulvodynia was superficial dyspareunia (p<0.0001). In some women the pain was also present during cycling or when using tight clothes. The symptoms had been present for a mean of 6.1 years (range 0.75-20 yrs) in women with RVVC and the mean duration of coital pain in women with LPV was 7.4 (range 1-10) years.

Table I. Demographical data, body mass index, results from a self-reported questionnaire

<table>
<thead>
<tr>
<th>DEMOGRAPHICAL DATA</th>
<th>Women with RVVC (n=57)</th>
<th>Women with LPV (n=41)</th>
<th>Control women (n=49)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>Mean (range)</td>
<td>Mean (range)</td>
<td>Mean (range)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>27.4 (19-38)</td>
<td>25.3* (21-36)</td>
<td>27.9 (18-39)</td>
</tr>
<tr>
<td><strong>Body mass index</strong></td>
<td>21.4 (17.1-24.9)</td>
<td>18.8†(17.8-23.8)</td>
<td>21.8 (18.1-29.0)</td>
</tr>
<tr>
<td><strong>Duration of present relationship (years)</strong></td>
<td>5.9 (0-14)</td>
<td>8.8 (0-15)</td>
<td>5.4 (0-15)</td>
</tr>
<tr>
<td><strong>Mean age at first intercourse (years)</strong></td>
<td>16.7 (11-23)</td>
<td>17.1(13-20)</td>
<td>18.8 (14-21)</td>
</tr>
<tr>
<td><strong>Use of oral contraceptives (years)</strong></td>
<td>7.4 (0-14)</td>
<td>8.3 (0-15)</td>
<td>5.6 (0-15)</td>
</tr>
</tbody>
</table>

* = significance level p< 0.05
† = significance level p< 0.005

Self-reported health questionnaire

Thirty-five percent of women with RVVC, 21.9 % of women with LPV and 40.1 % of the control women were current OC users. Fewer women with LPV had given birth than women with RVVC and controls (p<0.05). Approximately 70 % of the women with RVVC and LPV and 87 % of control women had a university level of education. Four women with RVVC, no woman with LPV and three control women were unemployed. None of the control subjects but five women with LPV and seven women with RVVC had seen a psychologist or a
physiotherapist for personal matters not related to RVVC or LPV. Two women with LPV but none of the women with RVVC or controls had been treated for depression.

The results of the self-reported questionnaire are presented in table II

**Table II. Results of the self-reported health questionnaire**

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>RVVC (% yes)</th>
<th>LPV (% yes)</th>
<th>Controls (% yes)</th>
<th>p-value RVVC</th>
<th>p-value LPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previous anti-candida treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>local treatment &gt;3 times</td>
<td>94.7</td>
<td>18.8</td>
<td>16.3</td>
<td>&lt;0.0001*</td>
<td>ns</td>
</tr>
<tr>
<td>oral fluconazole</td>
<td>64.9</td>
<td>56.3</td>
<td>20.4</td>
<td>&lt;0.0001*</td>
<td>ns</td>
</tr>
<tr>
<td>oral fluconazole, daily treatment 1-6 months</td>
<td>38.6</td>
<td>15.6</td>
<td>0</td>
<td>&lt;0.0001*</td>
<td>ns</td>
</tr>
<tr>
<td>SEXUALITY</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>first intercourse at age&lt; 15 yrs</td>
<td>14.0</td>
<td>15.6</td>
<td>4.1</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>&gt;4 intercourses/week</td>
<td>7.0</td>
<td>0</td>
<td>6.1</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>previous good experiences of sex</td>
<td>80.7</td>
<td>71.9</td>
<td>98.0</td>
<td>ns</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>orgasm</td>
<td>87.7</td>
<td>78.1</td>
<td>89.8</td>
<td>ns</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>OC-user&gt; 3 yrs</td>
<td>73.4</td>
<td>59.4</td>
<td>63.3</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>History of genital infections</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bacterial vaginosis</td>
<td>40.4</td>
<td>25</td>
<td>10.2</td>
<td>&lt;0.0005*</td>
<td>ns</td>
</tr>
<tr>
<td>condyloma</td>
<td>36.8</td>
<td>31.2</td>
<td>4.1</td>
<td>&lt;0.0001*</td>
<td>=0.001</td>
</tr>
<tr>
<td>genital herpes</td>
<td>1.7</td>
<td>0</td>
<td>0</td>
<td>=0.01*</td>
<td>ns</td>
</tr>
<tr>
<td>chlamydia</td>
<td>19.3</td>
<td>12.5</td>
<td>4.1</td>
<td>&lt;0.01*</td>
<td>ns</td>
</tr>
<tr>
<td>Hygiene habits</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>panty liner</td>
<td>7.0</td>
<td>43.8</td>
<td>4.1</td>
<td>ns</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Eczema/asthma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eczema</td>
<td>21.1</td>
<td>25</td>
<td>12.2</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>allergy</td>
<td>22.8</td>
<td>12.5</td>
<td>14.3</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>asthma</td>
<td>24.6</td>
<td>34.4</td>
<td>20.4</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Gastrointestinal symptoms</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gases</td>
<td>59.6</td>
<td>31.2</td>
<td>14.3</td>
<td>&lt;0.0001*</td>
<td>&lt;0.005*</td>
</tr>
<tr>
<td>diarrhea</td>
<td>24.6</td>
<td>15.6</td>
<td>4.1</td>
<td>&lt;0.005*</td>
<td>&lt;0.005*</td>
</tr>
<tr>
<td>constipation</td>
<td>26.3</td>
<td>6.2</td>
<td>10.2</td>
<td>&lt;0.05*</td>
<td>ns</td>
</tr>
<tr>
<td>irritable colon</td>
<td>14.0</td>
<td>12.5</td>
<td>0</td>
<td>&lt;0.01*</td>
<td>&lt;0.05*</td>
</tr>
</tbody>
</table>

*= significance level p<0.05, Mann Whitney U test
To summarize, previous anti-candida treatment and a history of genital infections such as bacterial vaginosis, condyloma, genital herpes and chlamydia was more common in women with RVVC, compared with controls. More women with LPV reported a history of condylomata and had more difficulties with libido and orgasm, compared with the control women. Use of panty-liners was more common in women with LPV, compared with women with RVVC and controls. A history of headache was reported more often in women with LPV, compared with women with RVVC and controls. No other differences in pain from other parts of the body were registered in our material. Gastrointestinal symptoms were more common in both women with RVVC and in women with LPV.

**Morning awakening salivary cortisol**

*Paper I and V*

Blunted morning rise cortisol was present in more women with RVVC and LPV than in control subjects (Fig 11). In women with RVVC mean levels of salivary cortisol (value at 15 min (p=0.01), 30 min (p=0.001), 45 min (p=0.01), value 1-4 (p<0.01) and value 1-5 (p=0.01), were significantly lower than in the controls. However, no significant difference was found between mean levels of cortisol in women with LPV and controls. Neither did the mean levels of salivary cortisol at bedtime differ between the groups (Table III).

**Table III. Salivary cortisol pattern in women with RVVC, LPV and control women (nmol/l)**

<table>
<thead>
<tr>
<th></th>
<th>Mean wake up 15 min</th>
<th>Mean 30 min</th>
<th>Mean 45 min</th>
<th>mean bedtime value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RVVC</td>
<td>16.7</td>
<td>18.3*</td>
<td>22.8*</td>
<td>18.7*</td>
</tr>
<tr>
<td>LPV</td>
<td>14.7</td>
<td>19.3</td>
<td>22.6</td>
<td>21.1</td>
</tr>
<tr>
<td>controls</td>
<td>12.8</td>
<td>20.5</td>
<td>25.5</td>
<td>24.6</td>
</tr>
</tbody>
</table>

* = significant values, p<0.05
Figure 11. Morning rise cortisol in women with RVVC, LPV, and in control women

Self-reported internet-based stress questionnaire

*Paper II and V*

According to the self-reported questionnaire Docco®, women with RVVC as well as women with LPV reported more symptoms of stress, compared with the control subjects (Table IV).
Table IV. Results of the self-reported stress questionnaire Docco®

<table>
<thead>
<tr>
<th>SYMPTOMS (SCALE 1-4)</th>
<th>DOCCO (PERCEPTION mean value)</th>
<th>RVVC (n=33) mean value</th>
<th>LPV (n=33) mean value</th>
<th>CONTROLS (n=28) mean value vs. RVVC vs. LPV controls</th>
<th>P-VALUE</th>
<th>P-VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Situation at work</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>strain at work</td>
<td>2.30</td>
<td>2.30</td>
<td>2.15</td>
<td>ns</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>worrying factors at work</td>
<td>1.80</td>
<td>1.55</td>
<td>1.50</td>
<td>0.02*</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>perception of control</td>
<td>2.80</td>
<td>3.10</td>
<td>3.10</td>
<td>ns</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>social support</td>
<td>3.25</td>
<td>3.35</td>
<td>3.40</td>
<td>ns</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td><strong>Private situation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>private demands</td>
<td>2.00</td>
<td>1.90</td>
<td>1.95</td>
<td>ns</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>private situation</td>
<td>2.60</td>
<td>2.30</td>
<td>2.40</td>
<td>ns</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>earlier life events</td>
<td>1.60</td>
<td>1.60</td>
<td>1.60</td>
<td>ns</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>meaningfulness</td>
<td>2.80</td>
<td>3.00</td>
<td>3.15</td>
<td>0.03*</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>perceived balance between work and leisure</td>
<td>2.55</td>
<td>2.95</td>
<td>3.00</td>
<td>0.01*</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td><strong>Lifestyle</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sleeping disturbances</td>
<td>3.15</td>
<td>3.05</td>
<td>3.3</td>
<td>ns</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>physical exercise</td>
<td>2.80</td>
<td>2.65</td>
<td>2.85</td>
<td>ns</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>smoking</td>
<td>2.65</td>
<td>3.90</td>
<td>3.85</td>
<td>ns</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>alcohol</td>
<td>3.85</td>
<td>3.65</td>
<td>3.60</td>
<td>ns</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td><strong>Personality</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>type A personality</td>
<td>2.65</td>
<td>2.55</td>
<td>2.45</td>
<td>ns</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>type D personality</td>
<td>2.15</td>
<td>2.15</td>
<td>1.85</td>
<td>0.04*</td>
<td>0.04*</td>
<td></td>
</tr>
<tr>
<td>Feelings of hostility</td>
<td>2.50</td>
<td>2.15</td>
<td>2.30</td>
<td>ns</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td><strong>Symptoms of stress</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>behavioral symptoms of stress</td>
<td>1.75</td>
<td>1.85</td>
<td>1.75</td>
<td>ns</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>emotional symptoms of stress</td>
<td>2.45</td>
<td>2.35</td>
<td>1.95</td>
<td>0.003*</td>
<td>0.02*</td>
<td></td>
</tr>
<tr>
<td>bodily symptoms of stress</td>
<td>1.85</td>
<td>1.85</td>
<td>1.55</td>
<td>0.02*</td>
<td>0.005*</td>
<td></td>
</tr>
<tr>
<td>cognitive stress symptoms</td>
<td>1.95</td>
<td>1.75</td>
<td>1.80</td>
<td>ns</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>burnout</td>
<td>2.40</td>
<td>2.35</td>
<td>1.90</td>
<td>0.001*</td>
<td>0.004*</td>
<td></td>
</tr>
<tr>
<td>psychological tension</td>
<td>2.65</td>
<td>2.65</td>
<td>2.30</td>
<td>0.04*</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>listlessness</td>
<td>2.50</td>
<td>2.50</td>
<td>2.10</td>
<td>0.02*</td>
<td>0.01*</td>
<td></td>
</tr>
<tr>
<td>mental tiredness</td>
<td>2.15</td>
<td>2.10</td>
<td>1.90</td>
<td>ns</td>
<td>ns</td>
<td></td>
</tr>
</tbody>
</table>

* = significance level p<0.05, Students T-test
Analyses of hormonal levels in plasma

*Paper I*

Serum samples for analysis of hormones were obtained on days 5 to 11 of the menstrual cycle. No significant differences were observed between women with RVVC and controls regarding levels of HbA1C, SHBG, DHEA-s, TSH, cholesterol, testosterone or bioactive testosterone.

As expected, levels of testosterone ($p<0.005$), bioactive testosterone ($p<0.0001$) and albumin ($p<0.001$) were significantly lower in women using OC ($n=27$), compared with women not using OC ($n=43$). No other differences were seen.

Analyses of glucose in vaginal secretions and in plasma

*Paper III*

The median level of plasma glucose was 5.0 mmol/L before and 5.8 mmol/L after oral glucose tolerance testing. Concerning the levels of glucose in vaginal secretions, a prominent interindividual level was found (between 0.2 and 149.0 mmol/L). The median vaginal level was 5.2 mmol/L before and 3.7 mmol/L after oral glucose tolerance testing. Urinary glucose was analysed in 52 women (21 patients, 31 controls). None of the women had detectable levels of urinary glucose.

When comparing cases ($n=38$) and controls ($n=45$), no significant differences were seen regarding change in paired comparisons of vaginal or plasma glucose at fasting and two hours after oral glucose tolerance testing (Table V, VI), (Figure 12 a, b). Moreover, HbA1c (Table V), (Figure 12 c) and body mass index (Table V) did not differ between the groups. The use of oral contraceptives did not influence the results (Table VI).
**Table V. Univariate analysis of change in vaginal glucose during oral glucose tolerance test**

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>p-value (t-test)</th>
<th>p-value (U-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>B-HbA1C</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>45</td>
<td>4.2600</td>
<td>.37863</td>
<td>.353</td>
<td>.544</td>
</tr>
<tr>
<td>Cases</td>
<td>38</td>
<td>4.3289</td>
<td>.27402</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>83</td>
<td>4.2916</td>
<td>.33467</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Body mass index</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>45</td>
<td>21.952</td>
<td>2.2746</td>
<td>.437</td>
<td>.435</td>
</tr>
<tr>
<td>Cases</td>
<td>35</td>
<td>21.571</td>
<td>2.0075</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>80</td>
<td>21.785</td>
<td>2.1569</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Difference in vaginal glucose</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>41</td>
<td>4.4764</td>
<td>20.00395</td>
<td>.256</td>
<td>.229</td>
</tr>
<tr>
<td>Cases</td>
<td>38</td>
<td>-4.6554</td>
<td>5.06484</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
<td>-2.6384</td>
<td>14.86840</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Difference in plasma glucose</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>45</td>
<td>.5967</td>
<td>1.36965</td>
<td>.419</td>
<td>.429</td>
</tr>
<tr>
<td>Cases</td>
<td>38</td>
<td>.3500</td>
<td>1.39027</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>83</td>
<td>.4837</td>
<td>1.37624</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

There were no differences in paired comparisons between women with (n=52) and without OC (n=31), either in vaginal or in plasma glucose before and after oral glucose tolerance testing (Table VI).
Table VI. Multivariate analysis of change in vaginal glucose during oral glucose tolerance test

Dependent Variable: difference in vaginal glucose

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>2.302</td>
<td>1</td>
<td>2.302</td>
<td>.010</td>
<td>.921</td>
</tr>
<tr>
<td>HbA1C</td>
<td>245.250</td>
<td>1</td>
<td>245.250</td>
<td>1.053</td>
<td>.308</td>
</tr>
<tr>
<td>Difference in plasma glucose</td>
<td>3.000</td>
<td>1</td>
<td>3.000</td>
<td>.013</td>
<td>.910</td>
</tr>
<tr>
<td>Body mass index</td>
<td>487.663</td>
<td>1</td>
<td>487.663</td>
<td>2.094</td>
<td>.153</td>
</tr>
<tr>
<td>Category (cases/controls)</td>
<td>108.371</td>
<td>1</td>
<td>108.371</td>
<td>.465</td>
<td>.498</td>
</tr>
<tr>
<td>Oral contraceptives (OC)</td>
<td>55.386</td>
<td>1</td>
<td>55.386</td>
<td>.238</td>
<td>.627</td>
</tr>
<tr>
<td>Category * OC</td>
<td>144.296</td>
<td>1</td>
<td>144.296</td>
<td>.619</td>
<td>.434</td>
</tr>
</tbody>
</table>
Figures 12a,b,c. Comparison of plasma glucose, vaginal glucose and HbA1C between cases and controls

**Paper IV**

Twenty women participated in this pilot study (9 women with recurrent vulvovaginal candidiasis and 11 healthy, age matched controls). Altogether 80 measurements of glucose in vaginal secretions and 80 measurements of plasma glucose were made.

There were no differences regarding body mass index, plasma glucose, or glycosylated haemoglobin A1C between the groups. None of the participants had a family history of diabetes mellitus. Body mass index was less than 25 in all the participants. Cases were older (median 26.0) compared with controls (median 22.0). Women using oral contraceptives were younger (mean 23.5) than women not using oral contraceptives (mean 28.0). No participant was excluded due to vaginal growth of *Candida albicans* (n=3), since this did not change the results.

The level of glucose in vaginal secretions and in plasma did not differ between women with RVVC and controls (Figure 13 a, b) (Table VII).

When comparing women with and without oral contraceptives, it was observed that the vaginal level of glucose at fasting was higher in women using oral contraceptives (p<0.001) (Figure 13 c). Plasma glucose was higher in oral contraceptives users 60 minutes after intake of 75 g of glucose (p=0.005) (Figure 13 d). All other vaginal and plasma levels were comparable between the two groups at all other measurements.
Table VII. Mean values of vaginal glucose and plasma glucose before, and 30 and 120 minutes after intake of 75 of glucose.

<table>
<thead>
<tr>
<th>Sample</th>
<th>v-glu before OGGT (mM)</th>
<th>v-glu 30 min (mM)</th>
<th>v-glu 120 min (mM)</th>
<th>P-glu before OGGT (mM)</th>
<th>P-glu 30 min (mM)</th>
<th>P-glu 120 min (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients*</td>
<td>2.4</td>
<td>0.6</td>
<td>0.4</td>
<td>5.4</td>
<td>8.7</td>
<td>6.9</td>
</tr>
<tr>
<td>(n=9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>3.3</td>
<td>2.2</td>
<td>1.3</td>
<td>6.1</td>
<td>8.3</td>
<td>7.2</td>
</tr>
<tr>
<td>(n=11)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

v-glu= glucose in vaginal secretions, p-glu= glucose in plasma
OGTT= oral glucose tolerance testing
*patients = women with recurrent vulvovaginal candidiasis

Figures 13 a-b. Plasma glucose levels

Figures 13 c-d. Glucose levels in vaginal secretions
DISCUSSION

In the western world, as much as 5-8% of the reproductive female population suffers from RVVC [19] and 15% of women report a history of various types of vulvodynia [64]. These disorders are thus very common, and affect the quality of life and sexual relationships of millions of women worldwide. The pathogenesis is multifactorial and still, after decades of research, poorly understood. Moreover, there is an increasing number of women seeking help from various (often several) health providers for RVVC and LPV. In this thesis lifestyle factors such as chronic stress and intake of glucose and their role in the pathogenesis of RVVC and LPV are evaluated.

Women that suffer from RVVC and LPV have several characteristics in common. Up to 75% of women with LPV have a history of recurrent vulvovaginal candidiasis. Women with LPV and RVVC are young, generally 20-30 years old. The results of the self-reported health questionnaires in our studies do support an the theory that there is an association between RVVC or treatment for RVVC and LPV. More women with RVVC than controls reported a history of lower genital tract infections, such as bacterial vaginosis, condyloma, herpes genitalis, and chlamydia. Women with LPV more often reported previous local treatment for condylomata and vulvovaginal candidiasis (p<0.0001), which among other local irritant disorders and treatments in the vulvar area are suspected to contribute to the development of LPV.

Age at first intercourse, number of partners, practise of oral sex, and number of intercourses/week did not differ between the affected women and control subjects. However, fewer women with LPV reported previous satisfaction with their sex lives (p<0.01), compared with controls and women with RVVC, probably due to the fact that some women with LPV have difficulties with sexual arousal. Women with LPV reported more frequent use of panty-liners compared with women with RVVC and controls. The use of panty-liners may cause chronic irritation of the vulnerable vulvar mucosa and might thus contribute to the development of LPV. Women with RVVC may have followed the advice to avoid the use of panty liners, since a connection between RVVC and frequent use of panty-liners has been registered. The reported history of asthma, allergies and eczema did not differ between the groups. Certainly, some women are more susceptible to RVVC due to genetic factors [32].

The incidence of stress related disorders in the Swedish society has increased during the last decade, particularly among women [171]. In today’s society, women encounter many demands
in private life and at work or during their studies. Sexual demands on many young women, also reflected by changes in sexual behaviour, have been found to be increasing [172]. In contrast to earlier generations, women nowadays work nearly to the same extent as men, while still having the main responsibility for household and children. It has been shown among executive directors that after work, norepinephrine levels are lowered in men, while they are elevated in women [173]. Approximately 70 % of patients and 87 % of controls in our studies were university students or had a university degree. Most participants were in a stable relationship. The fact that the women were highly educated might increase the risk for long time stress. If our control women had been recruited from a population of women with lower social and educational level, the difference in signs of chronic stress might have been even more significant.

**Psychoneuroimmunology**

Long-term mental stress, induced by the individual lifestyle, will gradually reduce the responsiveness of the HPA-axis, which may impair the function of the immune system [102]. This situation may increase the susceptibility to an opportunistic organism such as candida. An association between chronic stress and an impairment of the immune response has been observed in animal studies [108]. Furthermore, it has been found that chronic stress will aggravate allergies and atopic eczema in children [97]. According to recent results, women who suffer a burnout due to straining working conditions show an enhanced inflammatory response, manifested as elevated levels of the cytokine TNF-alfa [174]. It is thus likely that long-term perceived stress might contribute to a reactivation of candida vulvovaginitis, which is in analogy with the accepted association between stress and reactivation of herpes genitalis [175]. The observation that more patients than control subjects reported previous episodes of bacterial vaginosis, herpes genitalis and condyloma indicates a reduced local immunity in women with RVVC.

The impact of stress and lifestyle has only partly been addressed before [66]. In our studies, two independent methods for detection of chronic stress have been used. All the patients demonstrated blunted morning rise cortisol during the first 45 minutes after awakening. Moreover, women with RVVC had lower mean levels of salivary cortisol 15, 30 and 45 minutes after awakening, further supporting long time stress. Mean levels of cortisol were also lower in women with LPV, but did not reach significance. The fact that women with RVVC and LPV also reported more symptoms of stress, compared with healthy controls of the same age and
educational level, supports the results of the analysis of morning rise cortisol. More women with RVVC than controls complained of an exhausting labor situation and feelings of impaired balance between work and leisure time. Type D personality was more common in women with LPV than in controls. The most significant symptoms of stress registered in our patients were burnout, bodily and emotional symptoms of stress.

**Perceived stress and lifestyle, society of today**

RVVC and LPV are conditions per se that may produce chronic stress. Repetitive vulvar itching and burning, or painful sexual intercourse may have a negative influence on the relationship. However, in our material, no differences were seen between patients and control subjects regarding social support, private demands, and present private situation, such as relationship, family life or economic situation. Neither earlier life events, which has been shown to produce stress reactions later in life [176], nor perceived control, a key factor in the development of job strain [177], differed between the groups. The observation that 20% of our patients but none of the controls had experienced mental stress supports earlier results showing that psychological morbidity was more common in women with RVVC [178]. Psychological morbidity has also been investigated in women with LPV. Both the presence and the absence of psychopathology have been reported [70-73]. According to clinical experience it is obvious that some women with LPV show signs of depression and anxiety. Women with LPV have been characterized as perfectionistic, cautious, careful, insecure and pessimistic [74, 76]. In our study, signs of type D personality, i.e. depressive and pessimistic traits with feelings of hopelessness and helplessness were more common in women with LPV [179]. Depression may cause hypo-responsiveness of the Hypothalamus-Pituitary-Adrenal axis [138]. It is very likely that depression may be secondary to such a severe pain syndrome as LPV. However, it is also possible that primarily depressed women are at greater risk for the development of a vicious pain cycle. Moreover, body mass index was lower in women with LPV than in controls, supporting the tendency of perfectionistic traits.

Women with RVVC and LPV reported a history of gastrointestinal symptoms more often than the controls (Table II). Earlier studies have shown that such psychosomatic symptoms are common in women who perceive stress [171].

Chronic stress may increase the perception of pain [102]. It has been discussed whether LPV could be part of a generalized pain syndrome or a somatization syndrome [72, 180]. A connection to fibromyalgia has also been suggested [181]. Pain from other parts of the body is
common in vulvodynia patients [65, 79], as well as generalized systemic hypersensitivity with lowered pain thresholds for various stimuli in extremities [80, 82, 182]. In many somatization disorders such as “unexplained” musculoskeletal and gastrointestinal pain, sensitization may be present at multiple levels within the nervous system. At a higher level, cognitive processing may result in increased attention towards painful stimuli [183]. In our material, more women with LPV than controls reported a history of headache, but there were no differences in pain from other parts of the body.

Chronic stress may be secondary to the pain syndrome in women with LPV. If that is the case, it is important to emphasize that both RVVC and LPV are disorders that will cause so much distress for the woman that it affects the diurnal rhythm of cortisol. This situation may have long-time consequences on the health of the woman.

**Glucose metabolism in women with RVVC, diabetic, immunology**

Candida grows in vitro in an environment rich in glucose [51]. Elevated levels of urinary glucose, arabinose, and ribose were registered in subjects with RVVC and ongoing vulvovaginal candidiasis [52]. Furthermore, it has been shown that overgrowth of bacteria and fungi may elevate oral glucose levels [163].

Little scientific evidence exists for the recommendation of a carbohydrate-free diet in order to avoid recurrent vulvovaginal candidiasis. Moreover, in clinical practise, this regime is rarely successful. In this thesis, a method to measure glucose levels in the vagina is presented. A similar procedure to collect vaginal fluid has been described in measuring penicillin and cotinine [184, 185]. To our knowledge, vaginal glucose levels in women with RVVC have not been measured previously. It is conceivable that measuring the glucose level two hours after intake of 75 g of glucose is representative of the dietary situation.

With the described technique of sampling vaginal fluid, we did not find any differences in change of glucose levels during oral glucose tolerance test were found between women with RVVC and control subjects. However, there was a prominent interindividual variation of glucose levels in vaginal secretions. One reason for this difference between the subjects might be due to glycolysis from varying amounts of glycogen stored in the vaginal epithelial cells [186].

We were unable to repeat the results by Donders et al. showing elevated plasma glucose levels during oral glucose tolerance testing in women with RVVC [158]. In contrast to our material body mass index in the Donders study was higher in women with RVVC (mean 23.5),
compared with the control group (mean 21.4). He admits that the differences in body mass index may account for the differences in glucose tolerance that were found [158]. Fifty percent of both patients and controls in our sample as well as in the Donders study were oral contraceptive users [158, 187]. The use of oral contraceptives in his sample might have had an additive effect on the plasma glucose level, since the body mass index of the participating women was higher than in our study. In our material oral glucose tolerance testing did not increase levels of glucose in vaginal secretions, although the plasma glucose level was as high as 10-14 mmol/l in five women. This finding suggests that the activation of vulvovaginal candidiasis is unaffected by elevated levels of glucose in the vaginal fluids.

Women using OC may have slightly elevated levels of plasma glucose and HbA1C [44]. In addition, it has been shown that women with OC suffer from recurrent vulvovaginal candidiasis more often than women not using OC [44]. However, in our sample, the vaginal glucose did not differ between women with OC, and healthy women or women with RVVC. Nor was there any difference between patients with OC and healthy women not using OC. The tendency of recurrences of vulvovaginal candidiasis in OC users, as well as in diabetic subjects and in pregnant women, may be due to immunological factors [162], and not to elevated levels of plasma glucose. Women with immunosuppressive treatment also easily get vulvovaginal candidiasis. A deficient local immune system might be the pathogenic factor in common in these disorders. Babula and coworkers registered that women with RVVC have got reduced vaginal levels of anticandidal factors in IL-4* homozygotes, which may increase susceptibility to RVVC, due to a deficient local innate immunity [32]. Moreover, Fidel and co workers recently registered an impaired innate immunity in women with RVVC in vitro, and several studies have shown a deficient immunity in diabetic subjects, especially the innate part [162, 188]. Chronic stress may impair the immune system, in particular the innate part.

Hormonal samples

According to previous studies, chronic stress may have an influence on the levels of steroid hormones and HbA1C. However, in our study, we did not register any significant differences between patients and controls, regarding levels of S-SHBG, S-testosterone, S-bioactive testosterone, S-DHEAs, S-cholesterol, S-TSH or HbA1C. As expected, in women using OC the levels of testosterone were lower than in women not using OC.
Treatment implications

An impaired barrier function of the sensitive vulvovaginal mucosa may increase the risk for candida activation. Vulvar dermatoses such as lichen sclerosus and lichen planus, and contact dermatitis may be superinfected by vulvovaginal candidiasis. Moreover, microtraumata due to repetitive cleansing of the vulvar area or frequent sexual intercourse may facilitate the activation of candida. Repeated local treatments with antymycotics and cortisone ointments may also induce soreness and chronic irritation (Figure 13).

Figure 13. Possible mechanisms involved in the pathogenesis of RVVC

Too short treatment periods with oral and local antymycotics may cause recurrences of vulvovaginal candidiasis, considering that the MIC-value for fluconazole is 72 hours. Furthermore, it is important to strengthen the barrier function by not using products that may increase the vulnerability of the vulvovaginal mucosa (see figure). Long-time treatment with oral antifungals and oil regime will help to avoid recurrences.
According to our results a carbohydrate-free diet cannot be recommended as a prophylactic measure for RVVC.

For women with LPV, an individualized combination of local treatments and counselling is recommended. If there is no improvement in spite of 6 months of interrupted OC use and repetitive topical lidocaine application, a treatment model including biofeedback and counsellors addressing stress and lifestyle may be helpful. Many patients improve their coping skills and report reduction of stress symptoms and pain after the treatment (unpublished data).

CONCLUSIONS

Recurrent vulvovaginal candidiasis and localized provoked vulvodynia are two common disorders in young women that seem to be associated with severe dysregulation of the HPA-axis, perceived burnout and emotional and bodily stress symptoms.

Lifestyle factors such as chronic mental stress may play a role in the pathogenesis of both RVVC and LPV.

For the first time glucose in vaginal secretions has been analyzed in a group of healthy women and in women with RVVC.

Oral intake of 75 g of glucose does not affect the levels of glucose in vaginal secretions, and thus probably not the pathogenesis of RVVC.

There are no differences between women with RVVC and controls concerning levels of glucose in plasma during and after oral glucose tolerance testing.

Plasma levels of glucose are slightly elevated 60 minutes after intake of 75 g of glucose in women using oral contraceptives, compared with women not using oral contraceptives.

There is no scientific support for the recommendation of a carbohydrate-free diet as a prophylactic treatment for vulvovaginal candidiasis.
APPENDIX

(Examples of questions from the stress questionnaire Docco®, consisting of altogether 20 pages)

**Health**
To what extent have you, during the past months, had any of the following symptoms? Please mark the answer that best describes your situation.

**Worries/anxieties.**
- Not applicable
- I don’t agree
- I partly agree
- I fully agree

**Abnormal irritation.**
- Not applicable
- I don’t agree
- I partly agree
- I fully agree

**Feeling of being rushed.**
- Not applicable
- I don’t agree
- I partly agree
- I fully agree

**Feeling that something unpleasant will happen.**
- Not applicable
- I don’t agree
- I partly agree
- I fully agree

**Emotional instability.**
- Not applicable
- I don’t agree
- I partly agree
- I fully agree

**Undetermined feeling of threat.**
- Not applicable
- I don’t agree
- I partly agree
- I fully agree

**Feelings of guilt.**
- Not applicable
- I don’t agree
- I partly agree
- I fully agree

**Feelings of hopelessness.**
- Not applicable
- I don’t agree
- I partly agree
- I fully agree

**Feelings of helplessness.**
- Not applicable
- I don’t agree
- I partly agree
- I fully agree

**Feelings of dejection.**
- Not applicable
- I don’t agree
- I partly agree
- I fully agree

**Feelings of meaninglessness.**
- Not applicable
- I don’t agree
- I partly agree
- I fully agree

**Cry easily.**
- Not applicable
- I don’t agree
- I partly agree
- I fully agree

**Passiveness.**
- Not applicable
- I don’t agree
- I partly agree
- I fully agree

**Indifference.**
- Not applicable
- I don’t agree
- I partly agree
- I fully agree

**Lack of engagement.**
- Not applicable
- I don’t agree
- I partly agree
- I fully agree

**Problems with learning.**
- Not applicable
- I don’t agree
- I partly agree
- I fully agree

**Abnormal forgetfulness.**
- Not applicable
- I don’t agree
- I partly agree
- I fully agree

**Empty headedness.**
- Not applicable
- I don’t agree
- I partly agree
- I fully agree

**Slow-wittedness.**
- Not applicable
- I don’t agree
- I partly agree
- I fully agree

**Can’t make decisions.**
- Not applicable
- I don’t agree
- I partly agree
- I fully agree

**Abnormal fatigue.**
- Not applicable
- I don’t agree
- I partly agree
- I fully agree

**Muscle discomfort.**
<table>
<thead>
<tr>
<th>Condition</th>
<th>Not applicable</th>
<th>I don’t agree</th>
<th>I partly agree</th>
<th>I fully agree</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stiffness/lack in neck or shoulders.</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Headache.</strong></td>
<td>Not applicable</td>
<td>I don’t agree</td>
<td>I partly agree</td>
<td>I fully agree</td>
</tr>
<tr>
<td><strong>Palpitations.</strong></td>
<td>Not applicable</td>
<td>I don’t agree</td>
<td>I partly agree</td>
<td>I fully agree</td>
</tr>
<tr>
<td><strong>Pressure in the chest.</strong></td>
<td>Not applicable</td>
<td>I don’t agree</td>
<td>I partly agree</td>
<td>I fully agree</td>
</tr>
<tr>
<td><strong>Difficulty in breathing.</strong></td>
<td>Not applicable</td>
<td>I don’t agree</td>
<td>I partly agree</td>
<td>I fully agree</td>
</tr>
<tr>
<td><strong>Sweats.</strong></td>
<td>Not applicable</td>
<td>I don’t agree</td>
<td>I partly agree</td>
<td>I fully agree</td>
</tr>
<tr>
<td><strong>Dry mouth.</strong></td>
<td>Not applicable</td>
<td>I don’t agree</td>
<td>I partly agree</td>
<td>I fully agree</td>
</tr>
<tr>
<td><strong>Upset stomach.</strong></td>
<td>Not applicable</td>
<td>I don’t agree</td>
<td>I partly agree</td>
<td>I fully agree</td>
</tr>
<tr>
<td><strong>Nausea.</strong></td>
<td>Not applicable</td>
<td>I don’t agree</td>
<td>I partly agree</td>
<td>I fully agree</td>
</tr>
</tbody>
</table>

**Dizziness.**
I partly agree

**Diarrhoea.**
I partly agree

**Vision disorders, for example haze or spots before your eyes.**
I partly agree

**Comfort eating.**
I fully agree

**Excessive need of sleep.**
I fully agree

**Sleep poorly because of brooding.**
I fully agree

**Burnout**
I feel like I don’t want to go to work in the morning.

- **I'm physically exhausted.**
- **I feel that I have had enough.**
- **I feel full of energy.**
- **My “batteries” are drained.**
- **I feel alert.**
- **I feel burned out.**
- **I feel mentally tired.**
- **I feel like work in the morning.**
- **I feel active.**
- **I feel drowsy.**
<table>
<thead>
<tr>
<th>Feeling</th>
<th>Never/seldom</th>
<th>Fairly seldom</th>
<th>Very often</th>
<th>I feel very tense on the inside</th>
<th>Fairly often</th>
<th>Very often</th>
</tr>
</thead>
<tbody>
<tr>
<td>I feel tense.</td>
<td>Fairly often</td>
<td></td>
<td>Never/seldom</td>
<td>I can’t think clearly.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I feel relaxed.</td>
<td>Fairly often</td>
<td></td>
<td>Never/seldom</td>
<td>It is difficult for me to think about complicated matters.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I feel restless.</td>
<td>Fairly often</td>
<td></td>
<td>Never/seldom</td>
<td>My thoughts are fragmented.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I feel slow-witted.</td>
<td>Fairly often</td>
<td></td>
<td>Never/seldom</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Feeling</th>
<th>Never/seldom</th>
<th>Fairly seldom</th>
<th>Very often</th>
<th>I feel too tired to think.</th>
<th>Very often</th>
</tr>
</thead>
<tbody>
<tr>
<td>I have trouble concentrating.</td>
<td>Fairly often</td>
<td></td>
<td>Never/seldom</td>
<td></td>
<td></td>
</tr>
<tr>
<td>My thoughts are fragmented.</td>
<td>Fairly often</td>
<td></td>
<td>Never/seldom</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I feel slow-witted.</td>
<td>Fairly often</td>
<td></td>
<td>Never/seldom</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I feel slow-witted.</td>
<td>Fairly often</td>
<td></td>
<td>Never/seldom</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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