Psychoneuroendocrinological aspects of anorexia nervosa:

predictors of recovery

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General introduction

1

Anorexia Nervosa

Clinical description

Anorexia Nervosa (AN) is a complex psychosomatic eating disorder of unknown etiology, which primarily affects adolescent girls and young women (Hoek and Hoeken, 2003; Lucas et al., 1991; Lucas et al., 1999). It is characterized by aberrant patterns of eating behavior and weight regulation, which result in weight loss and endocrine abnormalities such as amenorrhea, in disturbances in attitude and perception about weight and shape, and in an intense fear of gaining weight. The Diagnostic and Statistic Manual 4th edition (DSM-IV, American Psychiatric Association, 1994) classifies patients as AN if they are incapable of maintaining a body weight above a minimum normal level (BMI < 17,5 kg./m^2 for adults), demonstrate an intense fear of becoming fat, have disturbed perceptions of body shape and size, and (after menarche) are amenorrheic. Two subgroups are recognized, the restricting type, in which weight loss is the result of dietary restriction, and the binge/purge type, in which periods of binging or purging and dietary restriction coexist. Compared to other psychiatric disorders, AN has the highest mortality rate, 10-15% (Sullivan ,1995).

Accompanying symptoms, such as perfectionism, obsessive-compulsive behavior, and social anxiety are observed in many but not all patients (Casper and Jabine, 1996; Piran et al., 1985). This characteristic cluster of personality and temperament traits often persists after recovery and has also been observed preceding the onset of disease (Halmi et al., 2003; Karwautz et al., 2003; Deep et al., 1995). In addition, a large proportion of AN patients displays abnormally high physical activity levels and overexercises, although due to definition differences, estimates vary, from 31 to 80% (Hebebrand et al., 2003).

Although the etiology of AN is as yet unclear, a combination of cultural, social, psychological, genetic and biological factors is implicated: "Genes load the gun, environment pulls the trigger" (Bulik and Tozzi, 2004).

In the Netherlands, the incidence of AN has been relatively stable over the last decade after an increase in the fifty years before that. In the period 1995—1999, the age and sex-adjusted incidence rate was 7.7/100,000. Interestingly, the incidence in female 15—19 year-olds (as well as in 10—14 year-olds) almost doubled when compared to 10 years earlier,

indicating that the onset of the disease is currently taking place at a younger age (Van Son, 2006). Average prevalence rates of AN in Europe appear stable at 0.29% of the total population (Hoek and van Hoeken, 2003).

Recovery from AN generally takes a long time and final outcome figures are regrettably low. Morbidity and mortality numbers are high; according to Steinhausen (2002), 20.8% (0—79%) of AN patients remain chronically ill, and 5.3% (0—22%) die either of starvation or suicide. Chances for recovery range from 0-92%, averaging at 46.5%. Data on younger patient are more positive, reaching about 60% for complete recovery (Steinhausen, 1997, 2002).

Historical perspective

The clinical syndrome of AN has been known for centuries, its core features largely unchanged since the first descriptions in the 19th century by Gull (1868) and Lasegue (1873). The conceptualization of AN however takes unexpected detours, only to reemerge in different countries and cultures. Dr. Christine Lafeber, in her 'Clinical-psychiatric study on anorexia nervosa' (thesis, 1963) describes AN as an example of how in medical circles new insights only emerge when the time is ripe.

The first clinical description of an anorectic patient dates back to the end of the 17th century, when Thomas Morton described a female and a male case in his 'Treatise on Consumption' (Silverman, 1985). Morbid self-starvation as a clinical syndrome started to receive attention 200 years later. In particular Gull described several case histories in which he emphasized the physical emaciation of anorexic patients as well as the sometimes remarkable degree of energy and activity exhibited, in light of their undernourished state. Lasegue went into more detail about the psychological state of his patients and their social interactions, particularly with members of their own family. Gull and Lasegue agreed on the clinical characteristics and an etiology that they considered psychological.

This picture held sway until 1914 when the pathologist Simmonds found lesions in the pituitary glands of some of his emaciated patients and AN became 'pituitary cachexia' or Simmonds' disease in which malnutrition was an endocrine disturbance involving the destruction of the pituitary gland (Sours, 1980). It took three decades for this erroneous notion

to disappear. After a phase in the 1930's when the concept of an endocrine deficiency caused by pituitary insufficiency combined with a psychological disorder (Ryle, 1936; Richardson, 1939) replaced its predecessor, the discussion of AN as a psychiatric disorder began anew.

After World War II the psychoanalytical approach dominated. Based on a publication by Waller et al. (1940) AN came to be understood as the symbolic expression of an internalized sexual conflict, with specific oral impregnation fantasies leading to fear of food intake. Treatment was based on the psychoanalytical interpretation of unconscious motives. Yet throughout the centuries AN as a disorder received only marginal attention unti the work of Hilde Bruch.

Hilde Bruch's work in the 1960's and 70's, changed the awareness of eating disorders and brought back a psychological approach (Bruch, 1973). Bruch emphasized transactions between the individual and her family environment, and focused on the 'relentless pursuit of thinness' and an 'all-pervasive sense of ineffectiveness' as concrete manifestations of the anorexic's failed quest for autonomy.

Later Bruch's exclusive focus on thinness, reflected in the new diagnostic formulations for AN in the Diagnostic and Statistic Manual (DSM-III, American Psychiatric Association, 1980) was questioned by observers from non-Western countries where body-image concerns did not seem central for many patients (Lee et al., 1993; Khandelwal et al., 1995). In this socio-cultural view, eating disorders were 'ethnic disorders', 'prescribed' templates for women in cultural transition to express psychological distress (Katzman and Lee, 1997). The psychoanalytic anthropologist Devereux (1980) expressed it this way: 'Don't go crazy, but if you do, do it this way.' Studies by Garfinkel and colleagues (1980a, 1980b) led to the hypothesis that strong cultural pressures to achieve thinness lead to high degrees of body dissatisfaction and dieting, and therefore make exposed women vulnerable to developing eating disorders. This seemingly paradoxical result was explained by feminist writers such as Wolf (1991) as an understandable consequence of gender politics, the demand for a 'reduction' of one's body size a response by the still male-dominated culture to women's assertion of their right to equal status in society. Garfinkel and Garner (1980b) were the first to link AN and romantic metaphors such as tuberculosis in the 19th century. In this view AN was a kind of perverse heroism of self-denial in a culture of plenty. AN in the 80's and 90's became glamorized and

associated with stardom. After an apparent increase in incidence of eating disorders during the second half of the twentieth century, numbers in the US and Europe seem to level off (Hoek and van Hoeken, 2003; van Son et al., 2006) and parallels were drawn with the rise and decline of conversion hysteria at the 'fin-de-siècle' (Gordon 1990). Meanwhile, countries such as China and India as well as Latin American nations show increasing numbers, which from a sociocultural viewpoint is not altogether surprising.

Finally, in the beginning of the 21st century there is a revival of interest in the biological and genetic origins of AN and other eating disorders, with the Price Foundation as a leading example.

Throughout the decades treatment has changed with the different explanations of the disease. However, treatment results of AN have not improved, not in the last place because there is still a serious lack of evidence-based treatment guidelines. And most unfortunate; treatment does not predict good outcome (Ben-Tovim et al., 2001).

Research

Since a combination of environmental, psychological, biological, and genetic factors is implicated in AN, researchers have focused on very different aspects of the disease. This section discusses the areas of research that have tried to unravel the processes underlying or maintaining AN.

Biology: Starvation

Anorexia Nervosa is characterized by the patient's refusal to maintain a minimal normal body weight and failure to respond normally to body weight loss. This results in a negative energy balance, which in turn disturbs the neuroendocrinological mechanisms that regulate appetite and food intake systems (Newman and Halmi 1988; Fichter et al., 1990; Levine, 2002). The consequences of the ensuing catabolic state are discernible on many levels in the central and peripheral hormonal feedback systems and associated organs (van Binsbergen et al., 1990; Kaye, 1996; Licinio et al., 1996; Berthoud, 2004; Mitan, 2004).

For decades scientists have tried to unravel the subtleties of the energy balance. Experiments in the 1940s on the effects of lesions in the hypothalamus led to the Dual Center Model of the regulation of feeding behavior, whereby the lateral hypothalamic area serves as feeding center, and the ventromedial area as the satiety center. In the 1950s Kennedy described the lipostat; the role of depot fat in the hypothalamic control of food intake, a theory about a fat marker (leptin) that was discovered 40 years later. In the following 3 decades there was a growing interest in the physiological abnormalities accompanying starvation and anorexia nervosa, and consequently a large number of papers describing the altered neuroendocrine parameters found in AN patients as compared to controls. With the discovery of leptin in 1994 (Zhang et al.) it became possible to link the alterations in peripheral and central parameters implicated in feeding behavior and to study the role of the neuropeptides involved. Recent leptin physiology research has established that the main role of this hormone is to signal energy availability in energy-deficient states. Studies in animals (Friedman and Halaas, 1998) and humans (Mantzoros, 1999; Ahima et al., 2000) have shown that low concentrations of leptin are fully or partly responsible for starvation-induced changes in the neuroendocrine axes, including declines in reproductive, thyroid, and insulin-like growth factor (IGF) hormone levels. Leptin is a peripheral satiety signal. It is mainly produced by adipose tissue, and is able to cross the blood-brain barrier to deliver its message to the hypothalamus. It is the principal long-term regulator of energy homeostasis. The role of leptin in energy balance regulation has been extensively studied (Friedman and Halaas, 1998; Ahima et al., 2000; Saper et al., 2002; Morton et al., 2006).

Activity and animal studies

A large proportion of AN patients (31—80% depending on the study methodology) exhibits abnormally high activity levels or hyperactivity, and overexercises (Favaro et al., 2000; Hebebrand et al., 2003; Holtkamp et al., 2006). The exact nature of hyperactivity remains to be clarified and although it is not included in the DSM-IV criteria, several authors, Gull being the first, (Hebebrand et al., 2003; Casper, 2006) have described and commented on this seemingly paradoxical phenomenon, which they see as a core symptom.

From the 1960s on, animal models that mimic human illness were developed. AN can be seen as the combination of weight loss and hyperactivity, and several experimental settings were developed to model this disorder, such as the activity based anorexia (ABA) model (Routtenberg et al., 1967; Kas et al., 2003) and the semistarvation-induced hyperactivity (SIH) model (Pirke, 1993; Exner et al., 2000). The ABA model is particularly widely used in animal studies on eating disorders. It consists of a combination of limited access to food (usually 1-2 hrs./day) and access to a running wheel. Although it is mostly used with rats as species, mice, guinea pigs, and hamsters react in the same fashion (Cornish and Morovsky, 1965). Rats exposed to the ABA model increase their total daily running wheel activity up to three fold; the natural distribution of activity throughout the day is also disrupted (Mistlberger, 1994). In the period before food is offered, rats develop substantial running wheel-activity and this activity finally even interferes with feeding, which seems to be crucial for the development of ABA (Dwyer and Boakes, 1997). ABA rats also show other 'symptoms' of AN, such as hypothermia and loss of an estrous cycle (Pare, 1977), and share several remarkable characteristics with AN-patients, such as gender distribution (Pare et al., 1978) and social isolation (Boakes and Dwyer, 1997). Female rats are more active in the model than males, and their reaction to the model is modulated by the estrous cycle, before this is disrupted (Eckel et al., 2000; Dixon et al., 2003). Age and initial body weight also play a role: young animals with a relative low body weight develop ABA faster than older, heavier animals (Boakes et al., 1999).

The SIH model differs from the ABA model in food availability. In SIH, rats are supplied with 60% of their base-line food intake but without a time restriction on when it can be consumed. Although in this model the rats' weight decreases also, it differs from the ABA model in the sense that in the latter although food is available, the rats do not consume it (Exner et al., 2000). Even while different, both models have been helpful in the search for a possible biological drive or changes in physiological parameters that trigger food restriction and hyperactivity.

Brain imaging

Brain imaging techniques that have evolved since the 1970s and 80s made possible another approach to understanding AN. Computer tomography (CT), positron emission tomography

(PET), single-photon emission computed tomography (SPECT), functional magnetic resonance imaging (fMRI) and magnetic resonance spectroscopy (MRS) have helped us to explore structural brain abnormalities, changes in regional blood flow, adaptations in neurotransmitter receptor binding, and alterations in brain chemicals.

The first imaging studies in AN were done in the 1980s; CT scans of AN patients revealed enlarged brain sulci and ventricles in combination with decreased brain mass (gray and white matter). These effects were reversed with weight gain (Dolan et al., 1988; Krieg et al., 1988). MRI studies later confirmed these results in ill patients, but while some showed normal brain tissue volumes after recovery (Wagner et al., 2006), other studies showed persisting abnormalities (Golden et al., 1996, Kornreich et al., 1991). Although the pathogenesis of the abnormalities is not fully clear, dehydration, loss of lean body mass, neuronal damage, and glucose levels play a role (Hendren et al., 2000). By means of MRS studies, which are able to show changes in brain metabolism, evidence has been found for altered membrane function, albeit with some controversial results. Schlemmer and colleagues (1998) hypothesized that these changes indicated an abnormal starvation-associated membrane turnover which occurs predominantly in the white matter. Roser (1999) could not confirm all of the findings but also found metabolic changes in the frontal white matter.

SPECT and PET analysis have revealed regional hypoperfusion and hypometabolism in the frontal and temporal-parietal lobe and hypermetabolism in the basal ganglia (Delvenne et al., 1999), some of which persisted after weight gain (Gordon et al., 1997; Rastam et al., 2001; Chowdhury et al., 2003). Weight gain seems associated with a normalization of regional cerebral blood flow (rCBF) in a number of brain areas, but the low level of rCBF in the arterior cingulated cortex (ACC) at baseline seems unaffected by treatment in restrictive AN patients (Kojima et al., 2005). Although these results are linked to eating pathology and starvation, the regions in which anomalies were found are also implicated in depression and anxiety, common comorbid symptoms of AN.

Another interesting finding comes from fMRI activation studies. The use of AN-provocative food-related stimuli results in increased activity in the left medial orbitofrontal cortex and anterior cingulated gyri and in decreased activity of the lateral prefrontal cortex, inferior parietal lobule and cerebellum, in both ill and recovered patients (Uher et al., 2003,

2004). Comparable results were found when patients rated body shapes which they perceived as aversive: the aversion ratings correlated positively with activity in the right medial apical prefrontal cortex (Uher et al., 2005).

PET scans using selective neurotransmitter ligands confirm the persistence of reduced 5-HT_{2A} in the frontal and parietal cortex and mesial and cingulated cortical regions after recovery from AN (Audenaert et al., 2003; Frank et al., 2002). This binding was associated with an anxiety measurement in women recovered from restricting-type AN. These data add to a growing body of evidence indicating that a dysregulation of serotonin pathways in cortical and limbic structures that may be related to anxiety, behavioral inhibition, and body image distortions (Kaye et al., 2005a). Frank et al. (2005) point to the dopamine function, they found increased D2/D3 receptor density in recovered AN women. This observation points to the possibility that there may be a dopamine-related disturbance in the reward systems contributing to altered hedonics of feeding behavior and the often present ascetic, anhedonic temperament. These psychobiological alterations may be trait-related and may contribute to the pathogenesis of AN. They also suggest new pharmacology and psychotherapy approaches (Bailer et al., 2005; Kaye et al., 2005b).

Psychology

Restricting-type AN in particular is characterized by a stereotypic cluster of personality traits found with remarkable consistency. These traits include perfectionism, harm avoidance, low self-esteem, obsessions, diminished self-directedness, and emotional restraint (Strober, 1995; Pryor and Wiederman, 1996); they persist after recovery (Srinivasagam et al., 1995) and also seem to precede the onset of the disease (Strober 1980; Pollice et al., 1997; Srinivasagam et al., 1995). Recent studies by Tchanturia et al. (2002, 2004) point to a related trait, rigidity, that is also of interest. Common personality disorders seen in AN are those in the so-called cluster C personality disorders (obsessive-compulsive, avoidant, and dependant) (Gillberg et al., 1995). This cluster of personality traits may be part of a genetically transmitted spectrum of temperamental risk factors for AN.

A different field of research interest focuses on the often observed comorbid symptoms depression and anxiety. The presence of these symptoms could be interpreted as genetic links,

as a comorbidity problem, or perhaps as AN with starvation leading to depressive or anxious states. A substantial number of clinical studies have demonstrated a link between AN and affective disorders (Hudson et al., 1983; Toro et al., 1995; Zonnevylle-Bender et al., 2004), and family studies have found an elevated lifetime rate of major mood disorders among relatives of individuals with eating disorders (Mangweth et al, 2003; Lilenfeld et al., 1998), with relative risks of 2 to 4.2. Although whether AN is a variant of an affective disorder or vice versa is still debated, the current consensus is that they frequently coexist but do not transmit together.
Anxiety disorders are very common in eating disorder patients (Deep et al., 1995; Braun et al., 1994; Smith et al., 1993). However very little data exist regarding the rate or pattern of transmission of anxiety disorders, except in the case of obsessive-compulsive disorder (OCD), in probands. Several family studies have established elevated rates of OCD with a lifetime risk of 3 to 4 for AN probands (Halmi et al., 1991; Lilenfeld et al., 1998).

The results of these several studies lead to the notion that there may be an AN subgroup with a genetic susceptibility to the so-called cluster C personality disorders and to OCD (Casper 1990; Halmi et al., 1991; Kaye et al., 2004).

Genetics

Last but not least, genetic studies have evolved over the years. First family studies, then twin studies, and, once techniques were developed in linkage and association, studies to identify vulnerability genes (Hinney et al., 2000), these studies all demonstrate that AN is not caused by a genetic variation within a single gene but is more likely the result of a complex interaction among multiple genes, and perhaps a genetic predisposition in combination with environmental and lifestyle factors.

Eating disorders cluster in families, the risk of probands of an AN patient to develop AN themselves is 2.68% vs. 0.18% in probands of unaffected controls; their relative lifetime risk 15 times higher (for overview: Gorwood et al., 2003).

Twin studies also show familial vulnerability, but concordance rates vary, depending on the population used (sometimes a general twin register such as used in the Virginia Twin Register studies, sometimes a population recruited in treatment centers). In a review of studies, Wade and colleagues (2000) conclude that shared environment does not play an important

role; heritability rates are estimated at 58%, with concordance rates of 0.55 in monozygotic and 0.05 in dizygotic twins.

Linkage studies investigate the prevalence of specific genetic markers in affected family members; association studies measure the differences in allele frequencies of the candidate genes between patients and controls. The first linkage studies did not produce significant results (Kaye et al., 2001) but subsequent reduction of sample heterogeneity and improved phenotyping, (e.g. by incorporating specific traits such as 'drive for thinness' and 'obsessionality') have yielded evidence for several chromosomal regions on chromosomes 1, 2 and 13 (Devlin et al., 2002). The linked region on chromosome 1 contains genes of the 5-HT_{1D}, δ-opioid, glutamate 7, orexin 1, and leptin receptors as well as the gene of the potassium channel hSKCa3. A combined linkage/association has confirmed the association with AN of the 5-HT_{1D} and δ-opioid, glutamate 7 receptor (Bergen et al., 2003), and also of the l hSKCa3 gene (Koronyo-Hamaoui et al., 2002).

Association studies can be done on positional genes (found under the linkage peak, see above) and on functional candidate genes. Association studies on functional candidate genes have resulted in polymorphisms in the serotonin system (5-HT_{2A}, $_{2C}$ and the serotonin transporter gene (SERT)), but other studies could not confirm these findings. Yet other studies looked into the systems that are implicated in body weight regulation and food intake, such as the orexigenic agouti-related protein (AGRP) (Vink et al., 2001; Argyropoulos et al., 2002; Marks et al., 2004; Dardennes et al., 2006), and the brain-derived neurotrophic factor (BDNF) gene (Ribases et al., 2003, 2005; Xu et al., 2003; Hashimoto et al., 2005;) but both our own group (de Krom et al., 2005) and Friedel et al. (2005) had negative results on BDNF.

Positive associations were also found in the gene cluster for uncoupling proteins (Campbell et al., 1999; Millet et al., 1997). Many other association studies have revealed negative or inconsistent results; further cooperation between geneticists and clinicians to refine phenotypes may contribute to more successful approaches in the future.

Longitudinal studies

Since the reports of Gull and Lasegue, AN has remained vivid in clinical thinking. Although Gull emphasized a 'central origin' and Lasegue a 'peripheral disturbance' as etiological factor, both agreed that emaciation without somatic pathology was characteristic of the disease. From this 'descriptive era' we arrived in the 'pituitary era' with Simmonds, followed by an 'era of rediscovery' in the 1930's, a 'psychoanalytic era' in the 1940s and finally a 'modern era' that by now has become a 'genetic era' and an understanding that AN results from a combination of cultural, social, psychological, genetic and biological factors.

Recovery from anorexia nervosa is often based on physical criteria, such as the return of menses and normalized bodyweight, but individuals considered to be physically recovered often continue to show distorted attitudes towards food, eating, and weight (Clinton and McKinlay, 1986). Psychological markers for physical recovery have not yet been found. Within the eating disorder literature clinicians have long recognized the importance of psychological dimensions of recovery from eating disorders (Bruch, 1974, 1982), but different definitions of overall recovery in different studies make comparisons difficult (Herzog et al.., 1999; Jarman and Walsh, 1999; Pike, 1998; Steinhausen, 2002; Strober et al., 1997). Most follow-up studies are based on the Morgan-Russell Global Assessment Score (MRGAS, Morgan and Hayward, 1988), which in turn is based on the Morgan-Russell Outcome Assessment Schedule (Morgan and Russell, 1975). Psychological aspects of recovery such as a reduction in fears about becoming fat, preoccupation with food and appearance, and disturbances in body image have historically received much less attention in outcome evaluations (van der Ham et al., 1994).

Recovery from AN takes a long time. Stable, continuous physical recovery is reached after on average 4.7 years and psychosocial recovery after 6.6 years (Strober et al., 1997; Fennig et al., 2002; Eckert et al., 1995). The final outcome figures of AN are unacceptably low. In his review of a large amount of outcome studies Steinhausen (2002) mentions that 20.8% (0-79%) of AN patients remain chronically ill and 5.3% (0-22%) die of starvation or by suicide (Steinhausen, 2002; Birmingham 2005). Chances for recovery differ greatly in the various studies from 0—92%, averaging 46.5%. Data in younger patient groups are more positive, reaching about 60% for complete recovery (Steinhausen, 1997, 2002).

On average 35—50% of patients experience one or more relapses, depending on the time frame of the study and the definition of relapse (Keel, 2005; Strober, 1997; Pike, 1998; Norring and Sohlberg, 1993). The often present comorbidity of anxiety and affective disorders influences outcome. Herpertz-Dahlmann and colleagues (2001) found a significant correlation after 10 years between psychiatric comorbidity and recovery from AN.

With the expanding knowledge of the biological underpinnings of AN and the growing possibilities to study them in animal models, questions arise about the legitimacy of correlating the two. Is the sequence of changes that result from prolonged starvation in humans with AN comparable to the changes observed in ABA rats? And what happens when patients start to gain weight? Are the biological changes observed linked to the psychological changes we observe?

Studies of biological parameters in humans are usually done with patients in the acute stage of the disease and with recovered patients; skipping the phase in which these changes are to occur. Many parameters that show abnormalities during starvation do indeed normalize upon weight recovery; others however remain abnormal or show an abnormal response to fasting. Several studies contradict each other (see Chapter 2). It is questioned to what extent AN patients show physiological reactions during a negative energy balance compared to normal controls, and whether they show maladaptation in certain physiological processes contributing to their pathophysiology. The main question remains why people treated to gain weight so often don't recover; or in other words, what keeps them from finding a way out of their unfortunate physical and psychological situation? Treatment has not become more successful over the years and predictions of who will succeed and who relapse are still very inaccurate on an individual level.

Aim of the thesis

This thesis aims to identify factors that predict or influence recovery outcome in AN.
Outcome studies show that the future for AN patients is rather poor (Steinhausen et al., 2002; Zipfel et al., 2000; Russell et al., 2001). Approximately 20–30 % of patients remain chronically

ill, another 30–40% reach an intermediate level sufficient to return to a productive level of functioning but with continued struggles around weight and shape. Only 40% recover with few sequelae. Treatment effect is often not lasting; patients may be discharged with a good treatment result, but the illness recurs within the first year after inpatient treatment in 30-50% of patients. Recent case-control studies have linked some biological factors to anorectic behavior; leptin levels at discharge for instance, seem to predict chances for recurrence of illness.

It was decided to study patients during a year of treatment, on their way to possible recovery, with close observation of both physical and psychological factors. Participants for this study were young girls and women consecutively referred to two specialized eating disorder treatment centers. The centers are geographically close, but one (at the Department of Child and Adolescent Psychiatry of the University Medical Center Utrecht) is for adolescents, and one (the Rintveld Center for Eating Disorders, Altrecht Mental Health Institute) is for adults.

Treatment of AN in these centers is based upon the American Guidelines of Eating Disorders after adjustment for the younger age of the patients (Robin, 1998; van Elburg and Rijken, 2004). After initial psychiatric assessment, patients entered a structured treatment program aimed at restoration of the patient's weight, normalization of eating patterns, body image, anorectic cognitions, and family and social functioning. Weight gain was targeted at 0.5–1.0 kg/wk in accordance with the guidelines. Weight recovery was defined as a weight within the normal range for age (> SD -1.5 corresponding with a Body Mass Index [BMI] of approximately 19 kg/m^2 for adults) and target weight as the weight at which patients resumed a regular menstrual cycle, defined as three menstrual periods at three to five week intervals. Individual, group, and family therapy techniques were used to change the patients' aberrant body perception and cognition.

Outline of the thesis

Chapter 2 describes the physical changes found over time during recovery. These include the changes in weight and body composition and the hormonal feedback loops that are influenced by leptin levels, the HPA-axis, the HPG-axis, the HPGH-axis and Ig-F.

Chapter 3 reports the results of our study of the validity and reliability of nurse observation of AN patient activity levels during treatment. Up to 80% of patients with anorexia nervosa manifest elevated levels of physical activity or hyperactivity. A variety of methods has been used to evaluate activity levels, mostly questionnaires but also expensive and invasive methods such as actometry or other measurements of energy expenditure. Nurse observations have heretofore not been tested for validity and reliability. We hoped to find that nurse ratings of activity correlated with actometer activity scores and also evaluated the ability of patients to rate their own activity levels.

Using nurse observations, the impact of hyperactivity and leptin on recovery from anorexia nervosa was explored (Chapter 4). In Anorexia Nervosa, high physical activity and low leptin levels are characteristic findings. Leptin, the hormone involved in the adaptation of an organism to starvation, has been linked both in animal and human studies to hyperactivity. We investigated the relationship between hypoleptinemia and hyperactivity in AN patients and assessed their predictive value for recovery.

We also looked at the effect of the atypical neuroleptic olanzapine on physical activity in anorexia nervosa (**Chapter 5**). The supportive effect of olanzapine in the treatment in AN patients has been described in several case reports. In this translational study we investigated the effect of olanzapine on hyperactivity both in patients and in rats exposed to the ABA model.

One of the important signs of recovery in anorexia nervosa is the resumption of a regular menstrual cycle. It is well known that while some AN women regain normal menstrual cyclicity immediately following body weight normalization, for other patients it can take additional months to years after the target weight has been reached. In **Chapter 6** we investigated the predictive value of various ovarian endocrine markers upon initial screening (especially AMH and inhibin B) for the resumption of normal menstrual cycles following weight recovery.

Psychological recovery in anorexia nervosa is reached on average 2 years later than physical recovery. In ***Chapter 7*** we describe the changes in mood states during recovery as measured by the POMS (Profile Of Mood States). We expected to find that physical recovery influences psychological recovery but is not sufficient to predict or cause recovery. ***Chapter 8*** contains a follow up study carried out about 5 years after the initial study.

The main findings of these studies are summarized and the clinical implications are discussed in ***Chapter 9.***

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Changes in body composition and hormone levels during recovery from Anorexia Nervosa

2

Abstract

Background: In Anorexia Nervosa (AN), food restriction leads to starvation which in turn causes changes in both central and peripheral hormonal feedback systems and associated organs. AN patients show physiological responses but also several unusual reactions in starvation conditions. In this study we investigated the changes in body composition and hormones implicated in eating behavior, menstrual cycle, and stress responsiveness in the acute stage of AN, and to follow alterations in these systems over a 9 month treatment period in patients who underwent a structured treatment program, aimed at restoration of weight, eating pattern, body image, normalisation of anorectic cognitions and family and social functioning.

Methods: Body weight and composition were measured at least once a week using a TANITA® body composition analyzer. Leptin, FSH, LH, estradiol, progesterone, testosterone, ACTH, insulin-like growth factor-1 (IGF-1) and cortisol were assessed biweekly in two AN patient groups (adolescents and adults) from the start and over the course of treatment, up to a year.

Results: Forty-two (69%) patients recovered in weight within the first year (WR), of which twenty four (39%) reached resumption of regular menstrual cycles (WCR); nineteen patients (31%) remained at a low weight throughout the year (NWR). The outcome groups initially differed in age (WCR group significantly older) and in estradiol levels, although all patients had levels clearly in the subnormal range. Body composition measures revealed significant fat mass differences among the three outcome groups. Leptin levels changed over time ($P < .01$), but the NWR changed significantly less than the other two outcome groups ($P < .01$). In the WCR group leptin levels normalized after approximately 4 months and a threshold was detected; an initial leptin level > 2 microgr./L increased chances for a full recovery to 75%.

For the HPG-axis hormones normal values were reached in the WCR group only. Cortisol normalized faster in the WCR group than in the other outcome groups but ACTH showed no change over time in any group. IG-F1 levels finally increased over time with the WCR group showing the highest (but still subnormal) levels.

Conclusions: Without normalization of weight, no normalization of hormones is to be expected. However, with a comparable and normalized weight WCR and WR groups show differences in leptin levels, fatmass and HPG-axis hormones. Rates of change between the NWR group and the other two outcome groups differ significantly for almost all hormone values. Between the WCR and WR groups significantly different rates are found for all HPG-axis hormones. These differences reflect the complicated nature of weight recovery during treatment for AN and the necessity to take body composition and changes in hormone levels, especially leptin in account.

Introduction

Anorexia Nervosa is characterized by the patient's refusal to maintain a minimal normal body weight and failure to respond normally to body weight loss. This results in a negative energy balance, which in turn disturbs the neuroendocrinological mechanisms that regulate appetite and food intake systems (Newman & Halmi 1988; Fichter et al., 1990; Levine 2002). The consequences of the ensuing catabolic state are discernible on many levels in the central and peripheral hormonal feedback systems and associated organs (van Binsbergen et al., 1990; Kaye, 1996; Licinio et al., 1996; Berthoud, 2004; Mitan, 2004).

The physiology of starvation has become better understood through studies in healthy volunteers (Keys et al., 1950) and through animal models (Elmquist et al., 1999; Morton et al., 2006). AN patients share the physiological responses of normal control subjects in starvation conditions including decreased plasma glucose levels and increased degradation of adipose tissue to generate energy, ketone body production in the liver to supply the central nervous system and the kidneys with fuel, and gluconeogenesis instead of glucose storage (Russell et al., 2001; Gold et al., 1986).

AN patients however also show several unusual responses including premature meal termination during refeeding and the often observed symptom of hyperactivity that leads to a paradoxical increase in total energy expenditure during caloric restriction (Casper et al., 1991). Controversial findings have been reported in AN patients regarding increased diet-induced thermogenesis (Casper et al 1991; Russell et al., 2001; Stordy et al., 1977) and regarding substrate utilization and body metabolism, which is lower than can be explained by body weight loss alone. This observation suggests reduced activity of metabolic tissue (Polito et al., 2000; de Zwaan et al., 2002). In AN patients some starvation induced changes, i.e. raised serotonin metabolite levels in liquor (Brown et al., 2003; Kaye, 1991) and abnormal the 5HT2A receptor levels (Kaye et al., 2001), fail to normalize with weight restoration, which abnormalities may be viewed as trait markers for eating disorders. Evidence of maladaptation in recovery has also been reported, including disproportional increases in leptin levels following weight gain (Hebebrand et al., 1997) or delayed resumption of normal menstrual cyclicity following body weight normalization, which can sometimes take additional months

to years after the target weight has been reached (Herpertz-Dahlmann et al., 1997; Golden, 1997).

The effects of starvation in normal individuals are powerful and long lasting too. Follow-up interviews with the participants of the ground-breaking Ancel Keys study in WWII revealed that 50 years later abnormal eating behaviors and ruminations persisted in almost all the men interviewed (Eckert & Crow, 9th Int. Congress on Eating Disorders, New York, 2000, unpublished data) . Recovery statistics for AN are disappointing: studies show that up to 50% of patients never recover and struggle lifelong with their drive for thinness, obsessionality (Devlin et al., 2002), and the perceptual and cognitive inflexibility (Tchanturia et al., 2004) that has been linked to constrained eating. Steiger and colleagues have linked trait variations to biology, especially serotonergic factors (Steiger 2004).

Most AN studies focus on measurements in the acute illness stage and after weight normalization. Very little research has been done into the process leading towards physical recovery. Indeed, it is not known whether recovering AN patients differ in the rate or the extent of change, or whether biological factors during the recovery process predict recovery. Therefore, we decided to study the changes in body composition and hormones implicated in eating behavior, menstrual cycle, and stress responsiveness in the acute stage of AN, and to follow alterations in these systems over a 9 month treatment period.

The biochemistry of starvation: the key role of Leptin

The central focus of this study is *leptin*, the protein product of the obese gene (Ob) discovered in 1994 (Zhang et al). Recent leptin physiology research has established that the main role of this hormone is to signal energy availability in energy-deficient states. Studies in animals (Friedman et al., 1998) and humans (Mantzoros, 1999; Ahima et al., 2000) have shown that low concentrations of leptin are fully or partly responsible for starvation-induced changes in the neuroendocrine axes, including declines in reproductive, thyroid, and insulin-like growth factor (IGF) hormones levels. Leptin is a peripheral adiposity signal. It is mainly produced by adipose tissue, and is able to cross the blood-brain barrier to deliver its message to the hypothalamus. It is the principal long-term regulator of energy homeostasis. Its role in feeding has been extensively studied (Friedman & Halaas, 1998; Ahima et al 2000; Saper et al., 2002, Morton et al 2006).

Leptin is part of the feedback system that maintains stores of body fat. A loss of body fat (starvation) leads to a decrease in leptin, which in turn stimulates a person to consume more calories than necessary for energy expenditure. Leptin also mediates a range of other metabolic and endocrine responses to fasting, changes that can be thought of as protecting against starvation: a decrease in reproductive hormones to limit procreation, a fall in thyroid hormones to conserve metabolic energy consumption, an increase in stress hormones (cortisol) to mobilize needed energy stores, and a rise in growth hormone with a decreased level of insulin-like growth factor-1, which releases alternative fuel stores through lipolysis (Ahima et al., 1996; Chan et al., 2003) (see Figure 1).

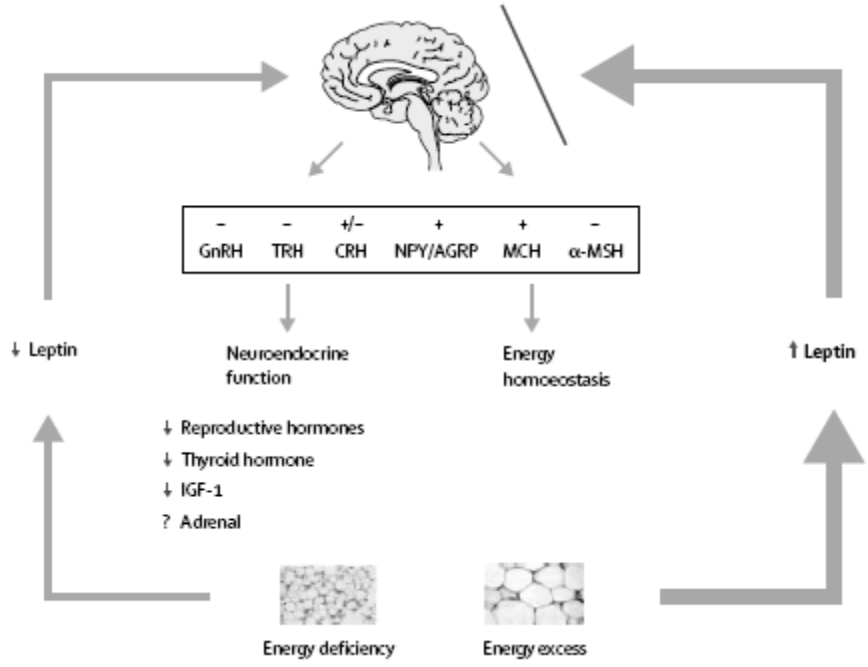


Figure 1: Physiology of the central effects of leptin in regulating neuroendocrine function and energy homeostasis in energy deficiency and energy excess (adapted from Chan & Mantzoros, Lancet 2005) GnRH= gonadotropin releasing hormone. TRH= thyrotropin releasing hormone. CRH= corticotrophin releasing hormone. NPY= neuropeptide Y. AGRP= agouti-related protein. MCH= melanin-concentrating hormone. α-MSH= α-melanocyte-stimulating hormone.

Evidence from genetic studies as well as behavioral animal work indicate that different hypothalamic neuropeptides mediate these responses (Saper et al., 2002; Ahima et al., 2000). The arcuate nucleus (ARC) of the hypothalamus contains at least 2 distinct neuronal populations regulating energy balance: the anorexigenic or appetite suppressing pro-opiomelanocortin (POMC) (Gee et al., 1983) and the cocaine- and amphetamine-regulated transcript (CART) (Douglas et al., 1996) on the one hand, and the orexigenic neuropeptides agouti-related protein (AgRP)(Hahn et al., 1999) and neuropeptide Y (NPY) (Schwartz et al., 2000) on the other (see Figure 2). Activation of the MC-4 receptor by its endogenous agonist, α-melanocyte-stimulating hormone (α-MSH), derived from POMC, is probably necessary for the biological response to increasing leptin levels and results in reduced food intake. CART, located in the same neuron, as well as the anorexigenic corticotropin-releasing hormone (CRH) (Uehara et al., 1998) also mediate some of leptin's effects in the hypothalamus. AgRP, the inverse agonist of the MC4R (Nijenhuis et al., 2001) and NPY, via binding to Y receptors, are the most important neuropeptides involved in the biological response to low levels of leptin and starvation (see Figure 2). Upregulation of these neuropeptides results in increased feeding behavior (Morton et al., 2006).

A multitude of neuropeptides and neurotransmitters thus regulate food intake, either in the brain or in the periphery. It appears that leptin is one of the most important regulators of food intake and that many of the above mentioned peptides act downstream of the leptin signaling pathway.

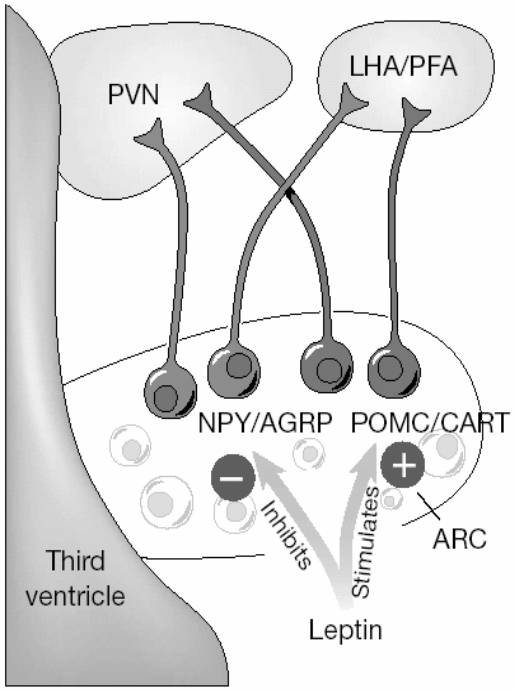


Figure 2 NPY/AGRP and POMC/CART neurons in the Arcuate nucleus (ARC) are regulated by leptin and project to the paraventricular nucleus (PVN) and to the lateral hypothalamic area (LHA) and perifornical area (PFA). (adapted from Schwartz et al., Nature 2000).

Several studies have shown significant correlations between leptin and body weight or fat (Monteleone et al., 2000; Kopp et al., 1998; Considine et al., 1996), but body fat percentage explains more of the variation in leptin levels (Mathiak et al., 1999). In AN patients in the acute stage, serum and cerebrospinal leptin concentrations are significantly lower than those of healthy controls (Hebebrand et al., 1997; Grinspoon et al., 1996; Mantzoros et al., 1997). The cerebrospinal fluid to plasma leptin ratio however, is higher in patients than in controls, and liquor leptin levels normalize early in the refeeding period. (Mantzoros et al., 1997). With weight gain, serum leptin concentrations increase rapidly and have been shown to reach higher levels than are seen in age- and weight-matched controls (Hebebrand et al., 1997). This effect has been observed to predict renewed weight loss (Holtkamp et al., 2004). Other studies have shown continued hypoleptinemia in weight recovered patients (Popovic et al.,

2004; Frey et al., 2000). Hebebrand et al. (2006) in a review on the role of leptin in anorexia conclude that AN leads to a long-term destabilization of leptin secretion, with both hyper- and hypoleptinemic periods. The clinical relevance of these alterations further depends on leptin receptor density and -mRNA splicing and on the quantity of soluble leptin receptors that are upregulated in AN patients (Monteleone et al., 2002; Misra et al., 2004; Kratsch et al., 2002) resulting in a reduction of free leptin.

Besides food intake, leptin also influences energy expenditure including thermogenesis, the menstrual cycle, growth, and possibly physical activity (see Chapter 4) and stress reactivity although studies in animals and humans show different results (Licinio et al., 1997; Heiman et al., 1997).

Leptin and reproduction (the HPG-axis)

Leptin receptors are expressed in the arcuate and ventromedial hypothalamic nuclei, specifically on gonadotropin-releasing hormone (GnRH)-secreting neurons regulating the release of gonadotropins (Yu et al., 1997; Cunningham et al., 1999). It has been suggested that leptin serves as a permissive signal to activate the reproductive axis (Kiess et al., 2000). Animal studies have revealed that leptin treatment accelerates the onset of puberty (Chehab et al., 1997; Ahima et al., 1997) or enhances sexual maturation in food-restricted rats (Gruaz et al., 1998). Leptin-deficient children, when treated with leptin, subsequently underwent puberty (Farooqi et al., 1998) and female patients with AN who gained weight as a result of dietary treatment showed a rise in leptin accompanied by a rise in luteinizing hormone (LH) and follicle stimulating hormone (FSH) levels (Audi et al., 1998; Ballauff et al., 1999). In AN, leptin levels drop because of food restriction and consequent weight and fat loss. As a consequence amenorrhea sets in, initiated by the negative feedback to the hypothalamus-pituitary-gonadal (HPG) axis and possibly also via direct effects at the level of the pituitary and ovaries (Chan & Mantzoros, 2001; Cioffi et al., 1997). The HPG-axis hormones progesterone, estradiol, FSH, and LH regulate reproductive tract functions during the menstrual cycle. In healthy fertile women, levels of these hormones fluctuate during the various phases of the menstrual cycle. Estradiol not only has a critical impact on reproductive and sexual functioning, but also affects other organs including bone structure. Low levels of estradiol

result in early osteopenia and osteoporosis, one of the serious consequences of AN. Estradiol is produced in the gonads from precursor hormones, specifically *testosterone*. In particular fat cells convert precursors to estradiol, and continue to do so even after the menopause. LH and FSH are secreted from cells called gonadotrophs in the anterior pituitary. Gonadotropin releasing hormone (GnRH) stimulates secretion of LH, which in turn stimulates gonadal secretion of the sex steroids testosterone, estrogen and progesterone. In a classic negative feedback loop, sex steroids inhibit secretion of GnRH and also appear to have direct negative effects on gonadotrophs. This regulatory loop leads to pulsatile secretion of LH and, to a much lesser extent, of FSH. Leptin accelerates GnRH pulsatility and may also act indirectly by altering the secretion of neuropeptides (Popovic et al., 2002). As noted earlier, leptin also may act on the pituitary directly; leptin receptors are expressed in ovarian follicular cells; and leptin-mRNA is expressed in human follicles (Cioffi et al., 1997).

Several studies have indicated that leptin levels must achieve a critical threshold or range for the HPG-axis to become active again (Kopp et al., 1997; Audi et al., 1998; Holtkamp et al., 2003). The gonads secrete at least two additional hormones whose relationship to leptin is as yet not fully clear but that may predict recovery of menses with weight gain. They are inhibin B and anti- Müllerian hormone (AMH), which selectively inhibit and activate gonadal function (Weenen et al., 2004; De Vet et al., 2002; Fanchin et al., 2003; Groome et al., 1996).

Leptin and the Hypothalamic-Pituitary-Adrenal (HPA) axis

The HPA axis has a central position in the stress response. Corticotropin-releasing hormone (CRH), the central component, stimulates the production of POMC gene products such as ACTH, which in turn stimulates the adrenal cortex to produce cortisol, the peripheral component of the HPA axis. Cortisol is essential for survival; without it, an organism is unable to respond to stress. The effects of cortisol can be divided into those that work on intermediary metabolism and those that work on water and electrolyte metabolism (gluconeogenesis, protein breakdown, increases in plasma glucose and insulin, body fat, suppression of immunity, maintenance of normal blood pressure and cardiac output).

Cortisol provides negative feedback to the HPA axis at the hypothalamus, the pituitary and the hippocampus; aberrant functioning can be characterized by abnormal levels

of HPA axis hormones or disturbances of circadian rhythmicity. In AN, increased central CRH, hypercortisolemia, and normal ACTH levels have been described with maintenance of the circadian rhythm (Licinio et al., 1996; Gold et al., 1986). Animal studies show increased corticosterone and ACTH concentrations under both starvation- and other stress-inducing circumstances, which reverses upon the administration of leptin (Ahima et al., 1996; Heiman et al., 1997). Human studies however, have established an inverse relation between fluctuations in leptin and those of adrenocotropin and cortisol. These could not be accounted for on the basis of glucocorticoid suppression of leptin but rather by a peripheral pulsatile leptin signal (Licinio et al., 1997). Another explanation could be that continued stress in the presence of decreased negative feedback to CRH production is secondary to decreased levels of the glucocorticoid receptor centrally, so that the pituitary production of ACTH is less inhibited. Cortisol is an important predictor of increases in trunk fat in adults with AN (Grinspoon et al., 2001) and recently Misra et al (2006a) showed that high baseline cortisol levels induced by increases in body fat predict menses recovery in AN.

Leptin and the Hypothalamic-Pituitary-Growth Hormone (HPGH) axis

Leptin receptors are present in the pituitary, and in vitro leptin increases GH secretion from pituitary cells (Giusti et al., 2002). In AN patients, especially the patients who are hyperactive, the HPA axis is activated resulting in hypersecretion of cortisol associated with normal ACTH levels (Loucks et al., 1989; Misra et al., 2004) and increased secretion of GH with lower IGF-1 levels (Misra et al., 2003, 2006). The HPGH-IGF1 axis stimulates proliferation and differentiation of osteoblast precursors and inhibits osteoclasts, but the effects of changes in metabolism in AN are not fully clear, especially in adolescents. Although studies have consistently reported low IGF-I levels in adolescents with this eating disorder, GH levels have been reported as low, high, or normal (Golden et al., 1994; Argente et al., 1997). Recently, Misra et al., (Misra 2006b) found an inverse relationship between BMI, fat mass, and leptin and GH secretion. They hypothesize that in underweight AN patients an acquired GH resistance occurs that is nutritionally regulated, and that the effects of nutrition exceed the effects of cortisol on GH concentration.

Aims of study

To increase our knowledge about the process leading towards physical recovery from AN, the rate or the extent of change, and the possible biological factors that can predict recovery process outcome by describing changes in body weight and fat, and hormone levels from the acute stage of AN through the first year of treatment.

Methods and materials

Subjects

Patients were admitted to this study after they and/or their parents gave informed consent. The clinical research protocol for this prospectively designed, cohort follow-up study was approved by the Ethics Review Committee of the University Medical Center of Utrecht (UMCU). Patients were recruited in two specialized eating disorder treatment centers (clinics), one for adolescents 12-17 years (UMCU, 31 subjects) and one for adults older than 17 years (Rintveld, 30 subjects). Inclusion criteria were (1) AN diagnosed according to DSM-IV (APA, 1994) on the basis of a structured interview using the Eating Disorders Examination (Cooper et al 1989), and (2) co-morbidity restricted to depression or anxiety disorders.,(3) secondary amenorrhea, (4) no use of steroid contraceptives, (5) exclusion of clinical histories of concurrent illnesses. Sixty-one female patients were included; 19 patients were of the AN purging type, 42 of the restricting type.

Study protocol

After initial psychiatric assessment, patients entered a structured treatment program, aimed at restoring weight, eating patterns, body image, and normalizing anorectic cognitions and family and social functioning. Weight gain was targeted at 0.5- 1.0 kg/wk in accordance with clinical guidelines. Weight recovery was defined as a weight within the normal range for age (> SD -1.5 corresponding with a Body Mass Index (BMI) of approximately 19 kg/m^2 for adults) and target weight as the weight at which patients resumed a regular menstrual cycle, defined as three menstrual periods with three to five week intervals. Individual., group, and

family therapy techniques were used to change the patients' aberrant body perception and cognition. Patients completed the study by achieving a regular menstrual cycle as described above, or after a year of participation.

Measures

Bodyweight & composition

Body weight and composition were measured at least once a week using a TANITA® body composition analyzer TBF-300 (Tanita Corporation, Tokyo, Japan). The degree of patient underweight was calculated using the body mass index (BMI, kg/m^2) computed into Z scores describing the statistical distance from the mean BMI for that age. Using a software program provided by the Netherlands Organization for Applied Scientific Research TNO, the data were related to Dutch population references (van Buuren & Fredriks, 2001).

Hormones

Blood samples were obtained by venepuncture for hormonal analysis at the time of admission and biweekly thereafter, at 4 pm. Plasma samples were stored at -80°C prior to analysis.

Leptin was measured using a sensitive Radio-Immuno-Assay (RIA) (Human-Leptin-RIA sensitive, Mediagnost, Tubingen, Germany), intra-assay CV of 5%, inter-assay CV of 7.6%.

FSH and LH were measured using a luminescence-based immunoassay (Centaur, Bayer, Tarrytown, USA) with a detection limit of 0.1 U/L. Intra-assay CV < 3% respectively 5% and inter-assay of 3.5-6% respectively 15%.

Estradiol was measured after diethyl ether extraction and Sephadex chromatography using an in-house competitive radioimmunoassay employing a polyclonal anti-oestradiol-antibody. [2,4,6,7-^{3}H]-Estradiol (TRK322, Amersham Nederland B.V.) was used as a tracer following chromatographic verification of its purity. The lower limit of detection was 20 pmol/l (2 mL sample) and inter-assay CV was 12 and 3% at 80 and 660 pmol/L (n = 45, respectively 25) respectively.

Progesterone was measured using a chemiluminescence-based immunoassay (Centaur, Bayer, Tarrytown, NY, USA) with a detection limit of 0.48 nmol/L, an intra-assay CV of 3 to 7% and an inter-assay CV of 2 to 6 %.

Testosterone was measured after diethyl ether extraction using an in-house competitive RIA employing a polyclonal anti-testosterone antibody (Dr.Pratt AZG 3290). [1,2-^{3}H(N)]-Testosterone (NET-387, DuPont NEN Nederland B.V.) was used as a tracer following chromatographic verification of its purity. The lower limit of detection was 0.12 nmol/L and inter-assay variation was 8,1; 5.6; and 6,6% at 0,77; 2,5 and 11,7 nmol/L respectively (n = 65).

ACTH was measured using an immunometric technique on an Advantage Chemiluminescence System (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). The lower limit of detection was 1.0 ng/L and inter-assay variation was 12; 7 and 5% at 5; 35 and 250 ng/L respectively (n = 40).

Insulin-like growth factor-1 (IGF-1) was measured using an immunometric technique on an Advantage Chemiluminescense System (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). The lower limit of detection was 6.0 ng/mL and inter-assay variation estimated over 1 year ranged from 6 to 8% in the range of 30 – 450 ng/mL.

Cortisol was measured using an immunometric technique (Advia Centaur, Bayer, Tarrytown, NY, USA) with a detection limit of <0.01 μmol/L in the range of 5.5 – 2069 nmol/L, an intra-assay CV of 3.5% and inter-assay CV of 4%.

Data analysis

After a follow-up of max. 1 year, the patients were divided into 3 groups according to their clinical status (Table 1). Data regarding clinical and endocrine parameters by group are presented as the mean ± standard error if distributed normally, otherwise as the median and range. Kruskal-Wallis tests were used if data were not normally distributed. If data were normally distributed, ANOVA was used. $P \leq 0.05$ was considered to be statistically significant. To account for the dependence between observations on the same person, a Linear Mixed Effects model was used, where necessary with a CAR(1) (continuous autoregressive of the 1st order) correlation structure and a power variance function. Hormone data (except

LH and IGF1) were transformed to better conform to a normal distribution. Square root transformation was used for estradiol, testosterone and ACTH; for all others a logarithmic transformation was performed.

Statistical analysis was performed using SPSS for Windows (release 11.5, SPSS Inc., Chicago, IL, USA) and S-plus software (Professional Edition 6.2, Insightful Corp., Washington DC, USA, 2003).

Results

Demographics

Mean age of the whole patient group was 18.2 ± 3.1 (SD) years, 15.9 ± 1.2 years for the adolescent group and 20.6 ± 2.9 years for the adult group. After a follow-up of maximum one year, the patients were divided into 3 groups according to their clinical status: no weight recovery (NWR), weight recovery but with ongoing amenorrhea (WR), and weight and cycle recovery (WCR). There were no significant differences among the three outcome groups in initial and premorbid body weight, duration of illness (defined by duration of amenorrhea), duration of study participation, or type of AN (restrictive or purging type) (Table 1).

Table 1. Demographics (mean + SD) of 61 young women diagnosed with AN. Data are presented for the entire group, and separately for women presenting with no weight recovery and amenorrhea (NWR), with weight recovery and amenorrhea (WR), and with weight and cycle recovery (WCR) during the study period (maximum 12 months).

Variables	Total group (n=61)	NWR (n = 19)	WR (n = 18)	WCR (n = 24)
Age (years)	18.2 ± 3.	17.3 ± 2.2	16.9 ± 3.2	19.8± 3.0* vs. NWR+ WR
Weeks in study	33.5 ± 11.4	32.8 ± 11.6	36.9 ± 14.0	31.6 ± 8.8
Bodyweight, premorb z scores	-.28 ± 0.96	-.34 ± 0.78	-.13 ± 0.98	-.33 ± 1.09
Bodyweight, initial z scores	-3.8 ± 1.6	-4.2 ± 1.9	-3.2 ± 1.5	-3.8 ± 1.2
Bodyweight initial (BMI)	15.5 ± 1.2	14.9 ± 1.3	15.7 ± 1.2	15.7 ± 1.0
Amenorrhea (months)	24.4 ± 22.8	20.5 ± 13.5	30.0 ± 30.5	23.9 ± 23.6
AN Restrictive type (%)	69	79	72	58

* $p < 0.01$

A difference in age was observed. The group that recovered in weight and resumed a menstrual cycle was significantly older than the non-recovered group. This result also corresponds with the clinic where they were treated, that is, recovery rates in the adolescent clinic were lower. Rates of recovering weight and menstrual cycle differed in the two clinics: 16% of the patients in the adolescent group recovered versus 63% of the patients in the adult group.

To be included in the study, patient participation had to be at least 3 months and ended at resumption of a regular menstrual cycle or a maximum of 12 months, whichever came first (see Figure 3). Menstrual status at 12 months was ascertained in 100% of the cases. Problems related to blood sampling, in combination with treatment protocol violations, turned out to be the main reason for premature dropout from the study. Based on the small number of remaining participants after these premature dropouts and of the patients in the WCR group at the end of the study, we decided to include data from the first nine months only.

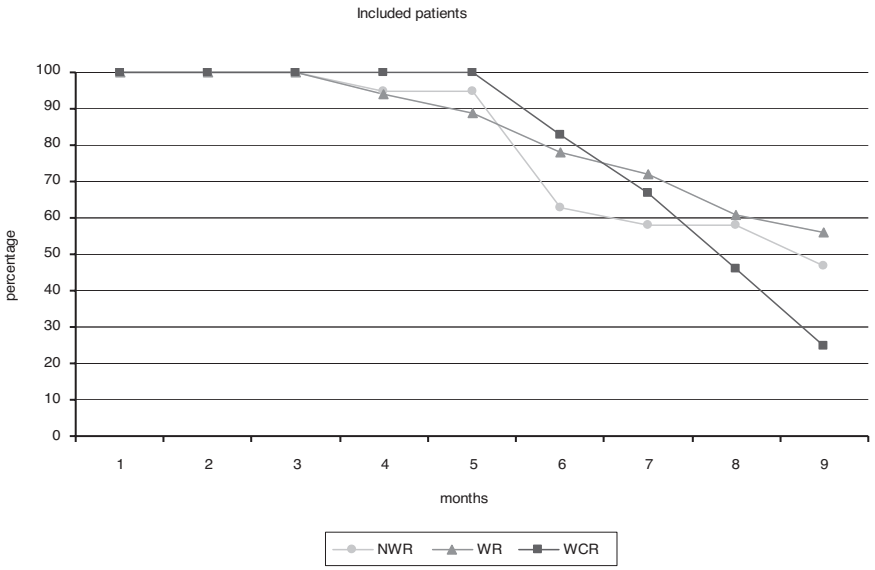


Figure 3. Percentage of patients participating in a follow-up cohort study of 61 young women diagnosed with AN. Data are presented for NWR, WR, and WCR over a period of 9 months. WCR patients ended study participation after resumption of menses, NWR and WR dropouts resulted from treatment protocol violations or problems related to the blood sampling procedure.

Body weight and composition

Average BMI at admission was 15.4 ± 1.3 kg/m^2 , which corresponds to a BMI Z score of -3.8 ± - 1.6 (Table 1). Within the 1 year time frame of the study, 42 (69%) patients met the weight recovery objectives, a BMI score of SD -1.5 or higher. Twenty-four (39% of the total, 57% of the weight recovered group) also recovered their menstrual cycles; 18 patients recovered in weight without the resumption of a menstrual cycle. Nineteen patients (31%) remained at a low weight throughout the year (see Figure 4, top).

Even though there were no differences in weight changes over time between the WR and the WCR group (P= .21), the body composition measures revealed significant fat mass differences among the three outcome groups (P< .001) with the WR group just barely reaching minimal normal values (Figure 4, bottom).

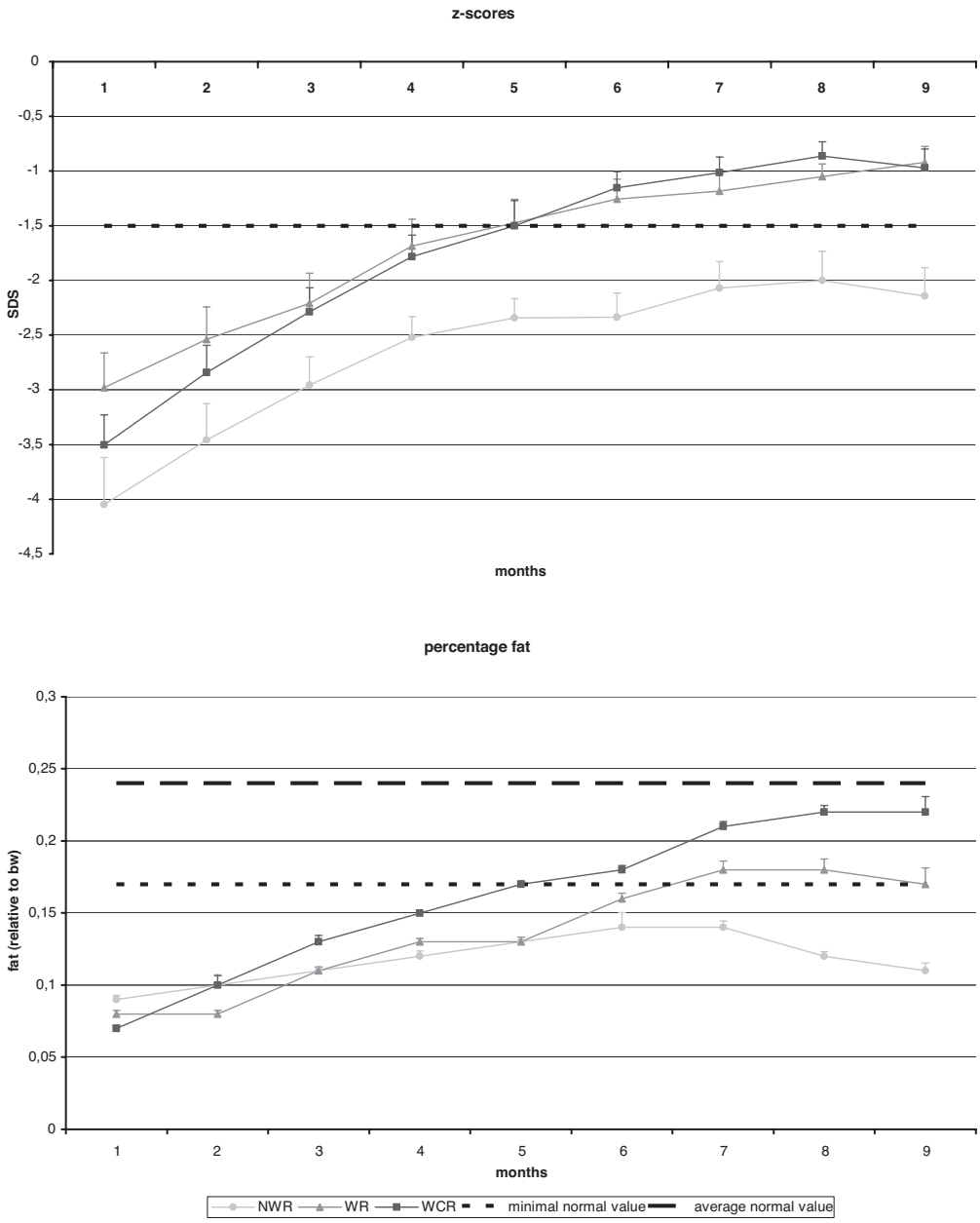


Figure 4. Body weight in BMI Z score (mean ± SE) (top panel) and percentage body fat (lower panel) of 61 young women diagnosed with AN participating in this follow up cohort study. Data are presented for NWR, WR or WCR over a period of 9 months.

Hormones

Table 2 shows initial values for all hormones, for the total group, and for each outcome group separately. The only significant initial difference was in estradiol levels, although all patients had levels clearly in the subnormal range.

Table 3 gives an overview of changes during recovery. With the exception of ACTH, all hormones showed significant changes over time.

Table 2. Initial endocrine characteristics (median and range) of 61 young women diagnosed with AN. Data are presented for the entire group and separately for NWR, WR or WCR.

Variables	Total (n=61)	NWR (n = 19)	WR (n = 18)	WCR (n = 24)
Leptin (µg/l)	2.1 (0.5-13.3)	2.9 (0.7-8.6)	1.5 (0.5-4.6)	2.4 (0.7-13.3)
LH (IU/l)	2.1 (0.3-16.5)	1.2 (0.9- 7.5)	1.5 (0.9-10)	3.1 (0.3-16.5)
FSH (IU/l)	2.8 (0.1-9.3)	2.8 (0.8-7.2)	2.0 (0.9-6.6)	3.8 (0.6-9.3)
Progesteron (nmol/l)	2.4 (0.4-11.0)	2.6 (0.5-4.3)	2.1 (0.4-4.2)	2.5 (0.4-11)
E_2 (pmol/l)	45 (19-260)	40 (19-120)	40 (19-130)	80 (39-260)* vs. NWR + WR
Testosteron (nmol/l)	1.0 (0.4-2.6)	1.0 (0.5-2.1)	0.8 (0.4-1.4)	1.2 (0.7-2.6)
Cortisol (µmol/l)	0.37 (0.12-0.86)	0.38 (0.13-0.85)	0.36 (0.12-0.59)	0.38 (0.14-0.86)
ACTH (ng/l)	22.0 (2-51)	22.6 (12-37)	19.7 (3-49)	20.1 (2-51)
IGF-1 (ng/l)	211.4 (75-434)	212,5 (92-352)	184,5 (75-434)	232.5 (146-384)

*$p < 0.05$

Table 3: Changes over time in hormones. Data are presented for the entire group and comparing rate of change between groups for NWR, WR, and WCR. All hormone data (except IGF-1 an LH) were transformed to better conform to a normal distribution. Square root transformation was used for estradiol, testosterone and ACTH, for all others a logarithmic transformation was performed.

Variables	Change over time	Interaction effects		
	Average of total group	WR – NWR	WCR – NWR	WCR – WR
Leptin	****	***	***	.07
FSH	****	****	.06	****
LH	****	ns	***	*
Estradiol	****	ns	***	*
Progesteron	****	ns	.05	**
Testosteron	****	**	*	ns
Cortisol	****	**	***	ns
ACTH	ns	ns	ns	ns
IGF-1	****	****	****	ns

* p <.05

** p <.01

*** p <.001

**** p <.0001

Leptin

Leptin levels changed over time (P < .01). The NWR differed significantly from the other two outcome groups (P< .01) (see Figure 5). The difference between WCR and WR groups almost reached statistical significance (P= .07). Absolute serum leptin levels of the WCR group normalized after approximately 4 months. Although levels of individual patients in the WCR group sometimes oscillated for a while, overall values remained within the normal range. The WR group continued to show subnormal serum leptin levels.

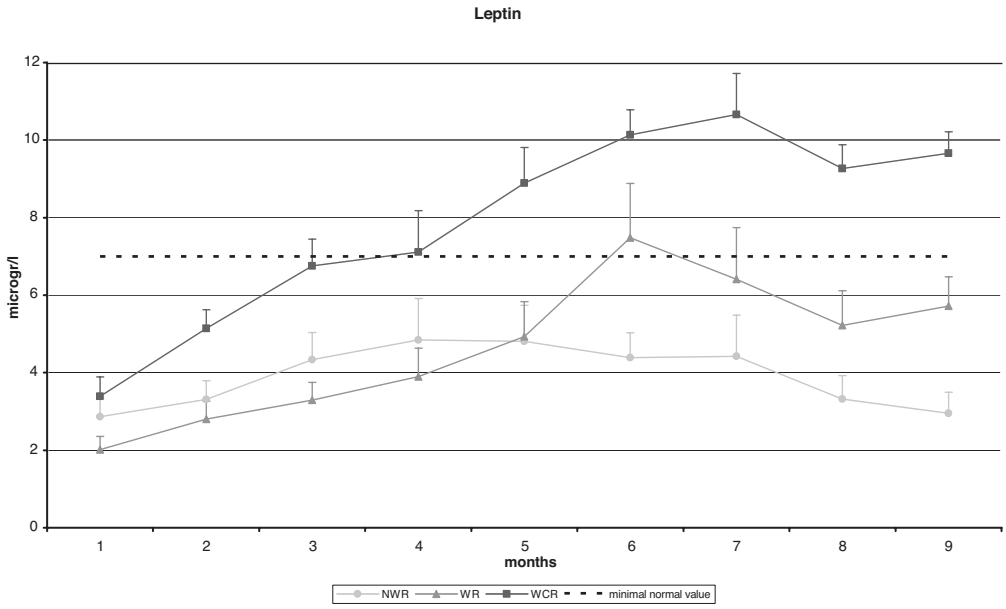


Figure 5. Serum leptin levels (mean ± SE) of 61 young women diagnosed with AN. Data are presented for NWR, WR, and WCR over a period of 9 months.

A leptin threshold level could be detected. To determine whether there was a threshold-like relationship between leptin levels and chances for recovery of ovarian function, we used a non-linear smoothing function in a Cox regression model stratified by clinic. Technically, a restricted cubic spline (a piece-wise third-order polynomial) with knots (change-points) at the P5, P35, P65 and P95 values of leptin was used (Harrell, 1988). Figure 6 shows the results for the adult group: an initial leptin level > 2 microgr./L increases chances for a full recovery to 75%.

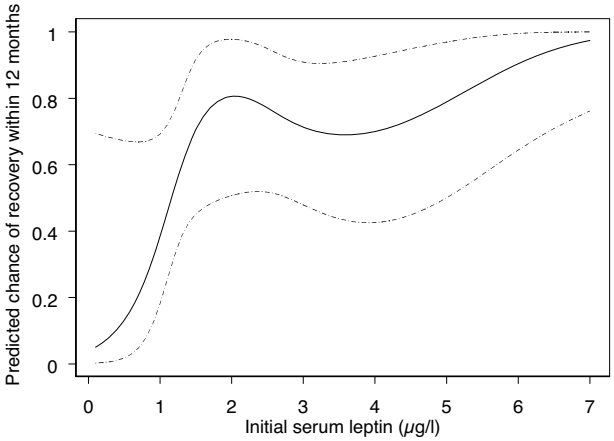


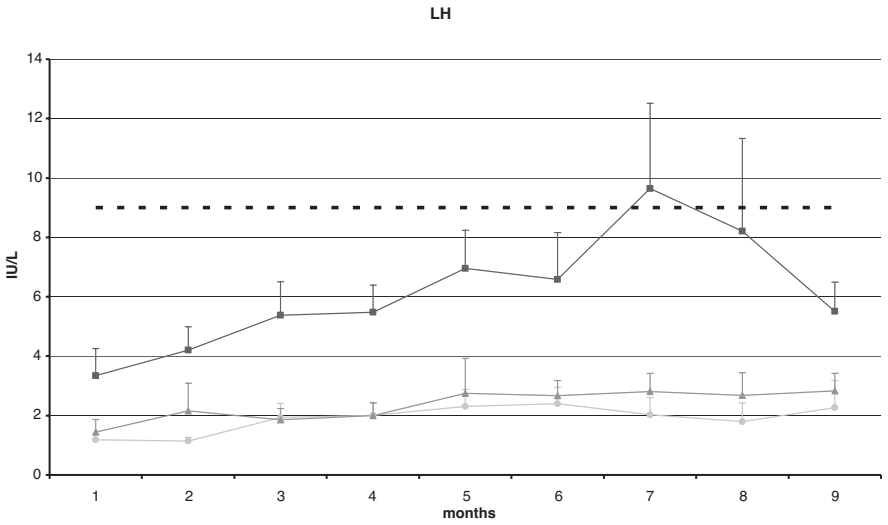
Figure 6. Association of initial leptin levels with chance of recovery of ovarian function during treatment to gain weight. Results shown are only for the adult patient group. Dotted lines represent 95% CI.

HPG-axis hormones

All HPG-axis hormones showed a significant change over time ($P < .01$).

LH levels of the WCR group changed significantly more over time than the other outcome groups ($P < .05$ and $< .001$ for WCR –WR and WCR-NWR resp.), between the WR and NWR group differences did not reach statistical significance ($P = 0.12$); only the WCR group reached serum levels sufficient for ovulation. FSH levels showed significant different changes over time between WR and NWR, and WCR and WR ($P < .01$). Progesterone levels changed significantly over time and WCR levels became significantly higher than the other outcome groups ($P < .01$). Again only in the WCR group did serum levels reach minimal values sufficient for ovulation. Estradiol changed over time. Initial levels in the WCR group were significantly different from the other two groups and also showed more change over time ($P < .01$ for NWR and $P < .05$ for WR) reaching ovulation levels. Testosterone levels remain in the normal range but changed over time, with a significant difference between NWR and WR/WCR ($P \leq .01$).

A


LH
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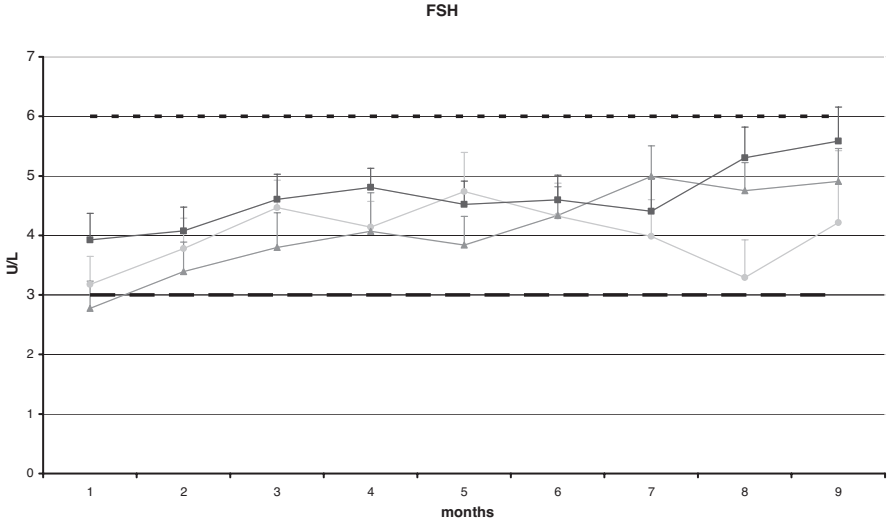
months

B


FSH
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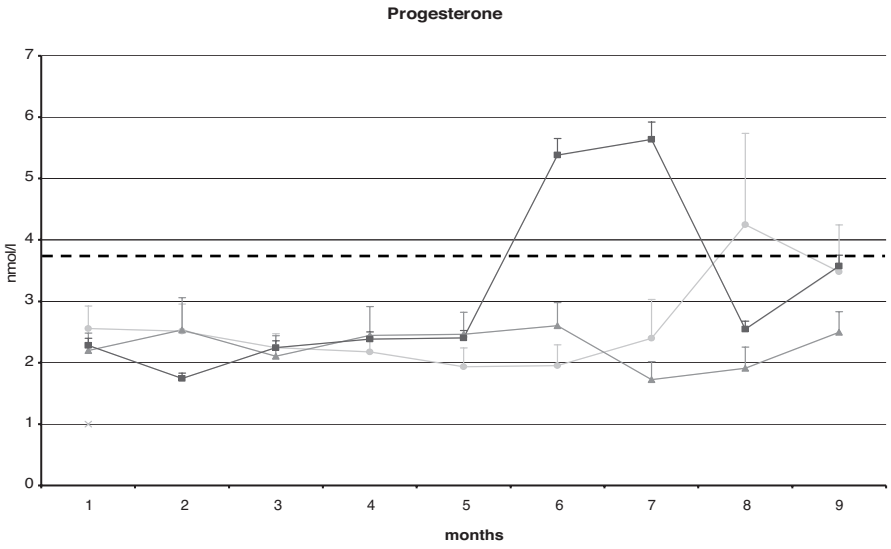
C


Progesterone
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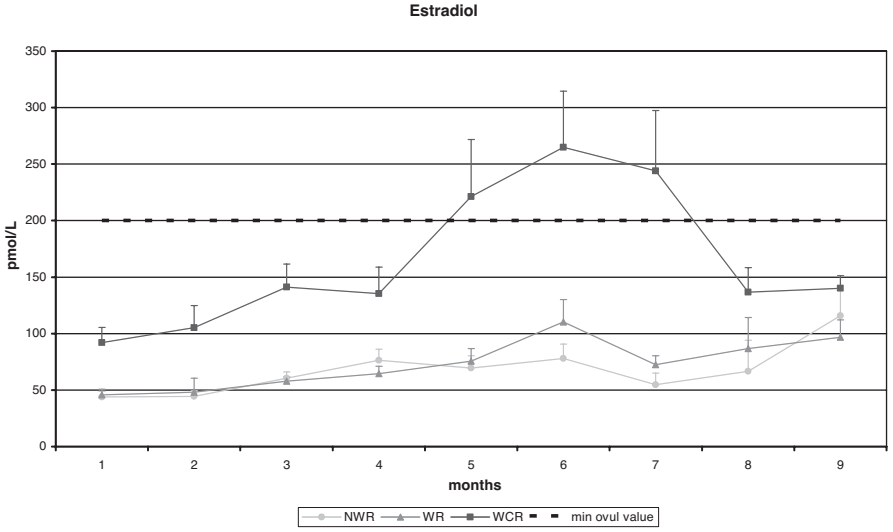
Estradiol
pmol/L
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NWR
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WCR
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E

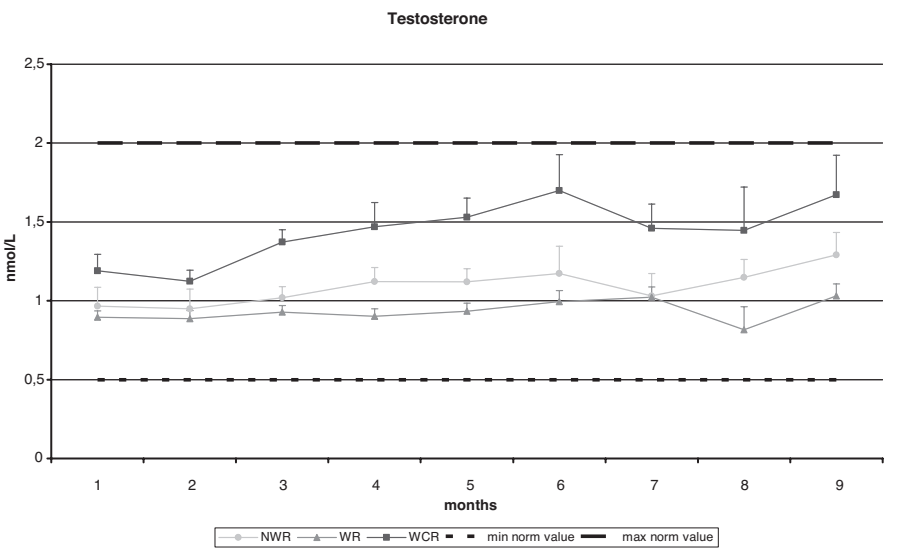

Figure 7 A – E. HPG axis hormones: LH, FSH, progesterone and estradiol, as well as testosterone (mean ± SE) of 61 young women diagnosed with AN participating in this follow up cohort study. Data are presented for NWR, WR or WCR over a period of 9 months.

HPA-axis hormones

Cortisol

As blood sampling occurred at 4 pm, measured cortisol levels indicate nadir values. Even so, initial cortisol levels were elevated and over time significantly different between the NWR and the WR and WCR groups ($P < .01$ and $< .05$ respectively). They normalized in all 3 groups within three months but the NWR group showed a worsening of levels in the second half of the study period.

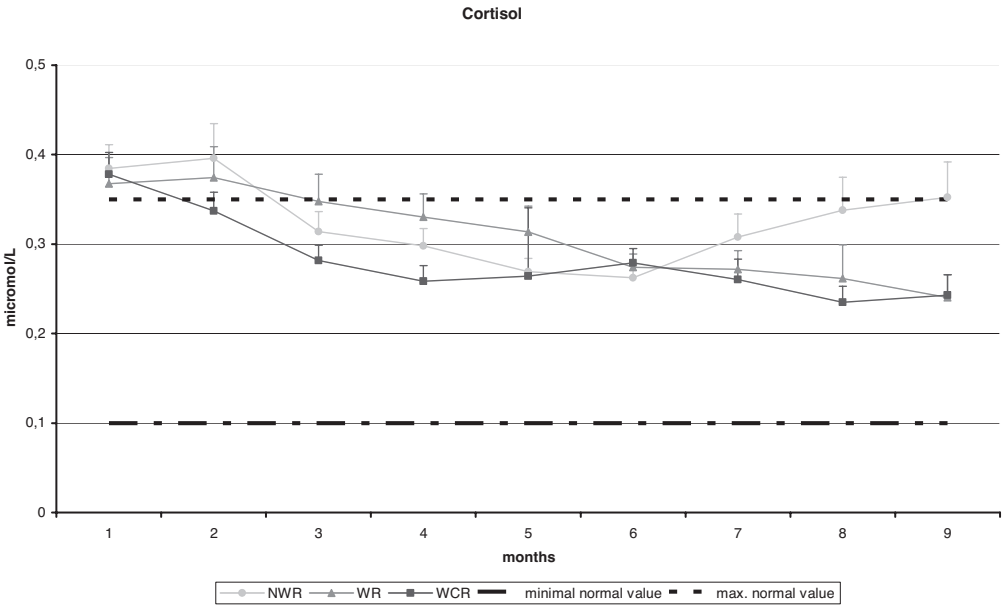

Figure 8. Cortisol (mean ± SE) of 61 young women diagnosed with AN participating in this follow up cohort study. Data are presented for NWR, WR or WCR over a period of 9 months.

ACTH did not change over time, nor were there any significant differences between the outcome groups (data not shown). All values were within the normal range.

HPGH-axis hormones

IGF-1

IGF-1 levels initially were decreased in all groups and changed significantly over time (P< .01), nearing normal values only in the WCR group, although between WR and WCR changes were not significantly different (P= .81).

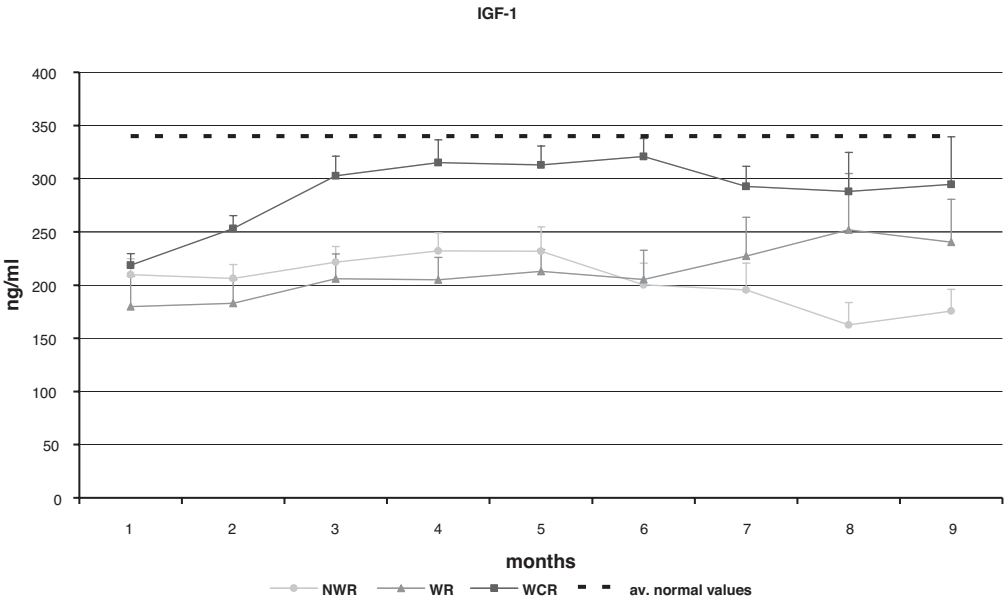

Figure 9. IgF-1 (mean ± SE) of 61 young women diagnosed with AN participating in this follow-up cohort study. Data are presented for NWR, WR, or WCR over a period of 9 months.

Discussion

In this study we were able to follow changes in weight and hormone levels during the first nine months of AN recovery. As could be expected, a considerable proportion of the patients, approximately 30%, gained very little or no weight, and remained at a weight level in the anorectic range (BMI <17.5 for adults). The other patients were able to gain sufficient weight to reach the normal range, defined as a weight higher than 1.5 SDS below average BMI for age, which corresponds with a BMI of 19 kg/m^2 for adults.

These results are consistent with the literature which indicates that the eventual outcome for AN patients referred to specialized treatment centers is rather poor (Zipfel et al., 2000; Russell et al., 2001; Steinhausen et al., 2002). Approximately 20 – 30% of patients remain chronically ill, 30 – 40% reach an intermediate level sufficient to return to a productive level of functioning but continue to struggle with weight and shape, and another 40% recover with few sequelae.

Resumption of the menstrual cycle represents an important step in the recovery of AN. This occurred in 39% of our patients within the first year. The exact extent of weight gain needed for full recovery of ovarian function is uncertain, but often a near 100% weight-to-height restoration is required, and persistent amenorrhea simply results from a relative subnormal weight. The difference in recovery chance between the two participating treatment centers can be explained in this way: at the start of the study the treatment program for adolescents (UMCU) had set a target weight at the 25th percentile of the growth curve, in line with publications (Hebebrand et al., 1996 a/b), but in many cases not sufficient for the resumption of the menstrual cycle. The adult clinic (Rintveld) used a target weight of > BMI 20.

While the 2 weight-restored groups WR and WCR achieved similar body weight, there were differences in body fat, leptin levels, and several HPG-axis hormones. Normal values were found in the WCR group only, consistent with a resumption of the menstrual cycle.

The reason why patients with comparable weight levels show such differences in body composition and consequently in hormone levels is not completely clear. Theoretically, there are several possible explanations. The origin may lie in differences in premorbid weight, resulting in the aforementioned relative subnormal weight. Higher activity levels could also account for differences in body composition after weight gain. Finally, differences in diet, or purging behavior may be involved. Premorbid weight however, did not differ between the outcome groups (see Table 1), and although activity levels are related to leptin levels in the acute stage of the illness, they did not correlate with longitudinal changes (see Chapter 4). Another explanation might be found in the amount of calories and more specifically the components of food consumed during weight gain. Although we know what diets were prescribed, there is no way to ascertain how much of it was really consumed. The outcome groups did not differ in the amount of patients with AN purging type. Last but not least, the differences in the 2 groups may reflect genetic differences. Animal studies into the melanocortin system using MC3 and MC4 knockout mice have shown that MC4 knock out mice become hyperphagic when fed a high fat diet, but that in the absence of hyperphagia, weight increased in the MC3 knockout mice as a result of a changing balance between activity and intake (Chen et al., 2000; Adan et al., 2006).

Almost all hormones changed over time with weight gain, with ACTH being the only exception to this rule. If normal values were reached this occurred in the WCR group only.

As was demonstrated before, patients in the acute stage of the illness showed elevated cortisol levels in combination with normal ACTH levels, confirming the findings by Licinio et al (1996). Recently, the presence of autoantibodies that bind to α-MSH or ACTH was demonstrated in AN patients, though not in vivo (Fetissov et al., 2002). Fetissov et al. describe several possible scenarios: disruption of the melanocortin signaling at the MC3 receptor, the MC4 receptor, or blockage of the α-MSH function to suppress the production of cytokines, which are potent inhibitors of food intake. A final possibility is serotonin binding, which would lead to interference with serotonin action and increased serotonin levels.

Of note in our study was the recurrence of high cortisol levels in the NWR group after several months. One explanation may be that these patients experienced a worsening of their stress and anxiety levels as time in treatment prolonged without positive results.

Prediction of outcome was possible with leptin and bodyweight (van Elburg at al., submitted). We could establish a leptin threshold with strongly rising chances for recovery up to a level of 2 micrograms per liter, increasing chances for a full recovery to 75%. Added to this result was the finding that the initial levels of the novel ovarian markers inhibin B and AMH, together with FSH, predict chances for a complete physical recovery within the first year (van Elburg et al., 2007).

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Nurse evaluation of hyperactivity in anorexia nervosa: a comparative study

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3

Abstract

Up to 80% of patients with anorexia nervosa manifest elevated levels of physical activity or hyperactivity. A variety of methods has been used to evaluate activity levels, mostly questionnaires but also expensive and invasive methods such as actometry or other measurements of energy expenditure. Nurse observations have heretofore not been tested for validity and reliability. In this study, 18 patients with anorexia nervosa under treatment in a specialized eating disorder centre simultaneously rated their own physical activity levels, used an actometer, and were observed for physical activity by trained nurses. We found that nurse ratings of activity correlated significantly with the average actometer activity score ($r= 0.61$, $p< 0.01$). Patients could not rate their own activity levels accurately. Nurse observation of activity levels of anorexia nervosa patients during treatment is a reliable and useful monitoring tool.

Introduction

A large proportion of anorexia nervosa (AN) patients (31-80 % depending on the criteria used and the study methods) exhibits elevated physical activity levels, described as hyperactivity, over-activity, motor restlessness and compulsive exercise (Favaro, Caregaro, Burlina, & Santonastaso, 2000; Hebebrand *et al.*, 2003; Holtkamp *et al.*, 2006). Almost all AN patients show a constant, agitated restlessness when they are emaciated, but before they become lethargic in the final stages of starvation. The different terms used to describe this elevated physical activity reflect different aspects or qualities of this symptom but also uncertainty about the origin of this behaviour that could reflect an altered state of mind and/or a neurobiological phenomenon and also questions about the extent to which patients deliberately reinforce the behaviour to reduce weight. In clinical practice an elevated physical activity level (hyperactivity) is a worrisome symptom. Hyperactivity leads to accelerated weight loss, to potentially lethal cardiovascular complications and because of its obsessive components often to dropout of treatment programs. The exact nature of hyperactivity remains to be clarified and although it is not included in the DSM IV criteria (American Psychiatric Association (APA), 1994), several authors (Hebebrand *et al.*, 2003; Casper, 2006) have described and commented on this seemingly contradictory phenomenon. Some authors state that it might be seen as a core symptom of AN (Casper, 1998). Neurobiological factors and conscious attempts to burn calories in order to loose more weight coexist. Hyperactivity has been described as a pre-morbid feature (Davis *et al.*, 1997) and it accelerates body weight loss during food restriction. Therefore measuring this behaviour is important for treatment, but also for identifying factors for genetic and behavioural susceptibility to AN.

In clinical practice a variety of methods has been used to evaluate hyperactivity: retrospective analysis of medical records (Crisp, Hsu, Harding, & Hartshorn, 1980), self-reports by means of activity diaries, experience sampling (Vansteelandt *et al.*, 2004), questionnaires (Slade, 1973) or self-ratings using visual analogue scales (Exner *et al.*, 2000), expert ratings using semi-structured interviews (Brewerton *et al.*, 1995; Davis & Kaptein, 2006; Davis, Kaptein, Kaplan, Olmsted, & Woodside, 1998; Davis, Kennedy, Ravelski, & Dionne, 1994) or scales of physical activity and motor restlessness (Holtkamp *et al.*, 2003), and devices such

as acto- and pedometers to measure movement (Blinder, Freeman, & Stunkard, 1970; Falk, Halmi, & Tryon, 1985). Although there has been no specific research on the reliability of hyperactivity self-reporting, many authors as far back as Gull (1888) have pointed out the paradoxical and ego-dystonic qualities of this symptom, even in children (Blinder *et al.*, 1970; Davis *et al.*, 1997; Fosson, Knibbs, Bryant-Waugh, & Lask, 1987). It remains uncertain to what extent patients consciously and deliberately exercise to continue to lose weight while their bodies already are wasted. Stone and Shiffman (2002) argue convincingly that self-reports in general are prone to error and bias because of the characteristics of autobiographical memory. Fichter and Quadflieg (2000) point out that comparing the reliability and validity of the self-report and the interview version of the Structured Interview for Anorexic and Bulimic Syndromes lower scores were found for items inquiring about hyperactivity. Both self-ratings and expert interviews soliciting patient recall often are retrospective and thus reflect the patient's memories of the months before the interview (Exner *et al.*, 2000; Holtkamp *et al.*, 2003). The correlation is therefore subject to the expert's ability to rate the patient's recall and prone to the forenamed bias.

As patients tend to exercise solitary, it appears difficult to estimate the quality and quantity of patient hyperactivity. Devices such as actometers measure motor activity mechanically and have been used with patient groups that do not exercise in private have been proven useful, with validity coefficients greater than 0.80 (Bouten, Westerterp, Verduin, & Janssen, 1994; de Vries, Bakker, Hopman-Rock, Hirasing, & van Mechelen, 2006). However the use of actometry has disadvantages. The devices require the cooperation of the individual, are uncomfortable for cachectic patients and are expensive as clinical diagnostic tools.

Because hyperactivity can be an important hampering factor in treatment of patients with eating disorders, and needs to be addressed specifically, we need to establish a reliable method to estimate and evaluate hyperactivity levels in clinical practice. Expert ratings of hyperactivity through observation have shown favourable results with psychiatric populations other than anorexia nervosa (Fitzpatrick & Donovan, 1979; Stevens, Kupst, Suran, & Schulman, 1978). More recently, nursery school teachers were found able to rate activity levels in young children reliably (Chen *et al.*, 2002).

To our knowledge, the reliability of nurse observations of hyperactivity in anorexia nervosa patients has not been tested. We decided to evaluate the reliability and validity of nurse observations of hyperactivity by comparing them to patient self-ratings and to actometer-measured activity levels. With the actometer results as the gold standard, we hypothesized that nurses would provide a more reliable and valid measure of hyperactivity than patient self-reports.

Method

Subjects

All 20 inpatients fulfilling DSM-IV criteria for AN and admitted during the course of the study to two specialized eating disorder treatment centres in the Netherlands (for adolescents in Utrecht and for adults in Zeist) were asked to participate. Patients were enrolled in the study after they, or in case of minors, their parents, gave informed consent.

Patients were able to move freely but were only allowed to remove the actometer when showering. If the data showed long periods without any activity (suggesting misuse of the actometer), patients were to be excluded from the study, this did not occur however.

Measures

Nurse rating

Two nurses were instructed to observe physical activity during three consecutive weekdays defined as the amount of motor restlessness (inability to sit still, moving arms or legs while seated, walking through the ward without reason), abnormal motor activity, and excessive exercise, and to score their observations on a visual analogue scale (0 -10).

Patient self-rating

Patients were asked to rate their own physical activity levels over the same days, in the same way as the nurses, using a similar visual analogue scale. They were also asked to rate their

mood states using the Dutch version (Wald & Mellenbergh, 1990) of the POMS (Profile Of Mood States), a questionnaire widely used in sports medicine research (McNair, Lorr, & Droppelman, 1971). The POMS contains, among others, items rating restlessness, feeling active, lively and tense.

Actometer

In the same observation period an actometer (Actiwatch, Cambridge Neurotechnology, Cambridge, United Kingdom) was strapped to the patient's right ankle, to measure physical activity levels during three consecutive weekdays, from 9 PM on the first day to 9 PM of the fourth day. This procedure was similar to that used by Holtkamp *et al.* (2006). The average activity score (Actiwatch Sleep Analysis 2001) was used for statistics.

Other measures

The degree of patient underweight was calculated using the body mass index (BMI, kg/m^2) computed into Z-scores describing the statistical distance from the mean BMI for that age. Using a software program provided by the Netherlands Organization for Applied Scientific Research TNO, the data were related to Dutch population references (van Buuren & Fredriks, 2001).

Results

Eighteen patients (mean age 17.9 ± 3.5 years) gave informed consent and participated in the study. All patients were underweight with an average BMI of 16 and a BMI Z-score of -3.0 (SD 1.6).

Linear regression analysis indicated that nurse ratings of activity correlated significantly with the average actometer activity score (Actiwatch Sleep Analysis 2001) ($r= 0.61$, $p=0.007$) (Figure 1). The ratings between the nurse raters showed a moderate correlation (Cohen's kappa $=0.57$, $p\leq 0.05$).

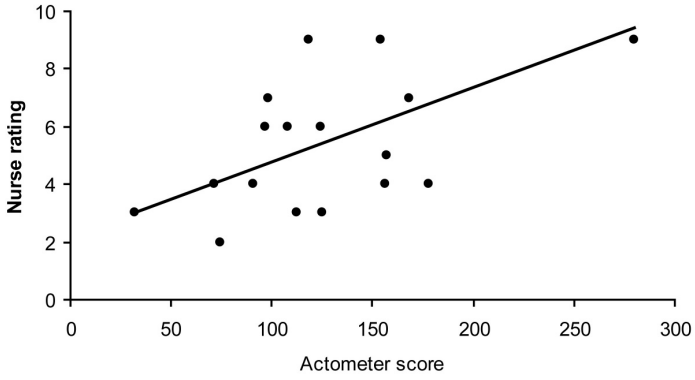

Figure 1. Nurse ratings of activity levels (defined as the amount of motor restlessness, abnormal motor activity, and excessive exercise) compared to actometer scores, r=0.61, p=0.007

Table 1 shows the correlations of the actometer scores with the self- and nurse ratings. Patient self-rating of physical activity on a visual analogue scale did not correlate with actometer scores (r= 0.44, p= 0.24). Also the POMS items restlessness, feeling active, lively, and tense did not show significant correlations with the actometer.

	Actometer score	
	Pearson Correlation	Sig. (2-tailed)
Nurse rating	,608	,007
Patient self-rating	,439	,238
POMS restlessness	,024	,927
POMS active	,471	,122
POMS tense	,148	,646
POMS full of energy	,128	,612

Table 1. Correlations between nurse ratings, patient self-rating (on a visual analogue scale and through questionnaire (POMS)), and actometer scores of activity levels in Anorexia Nervosa patients (N=18)

The physical activity ratings by nurses showed significantly higher results than the patient's self rating, 5.6 ± 2.5 versus 3.9 ± 2.1 respectively, $p < 0.01$.

Conclusion

This study shows for the first time that nurses can reliably rate activity levels of anorexia nervosa patients. The nurses' measurements of a patient's activity levels correlated with activity scores from actometers, the gold standard. Patient self-ratings did not correlate with the actometer scores nor did the use of a questionnaire to rate feelings of restlessness and activity improve the patients' ability to judge their own level of activity. Not surprisingly, patients rated their own level of activity lower than the nurses did, and lower than the actometer results showed.

Very few studies to date have compared self-ratings of activity with actometry measurements. Finn and Specker (2000) compared the Actiwatch with the Children's Activity Rating Scale, the results favouring the use of activity monitors; Rousham, Clarke, & Gross (2006) showed unreliable results for both compliance and correlation between the two methods in a group of healthy pregnant volunteers, and Smith *et al.* (Smith, Pelham, Gnagy, Molina, & Evans, 2000) showed that self-report of hyperactivity by ADHD patients is unreliable. Our findings confirm previous data from non-anorexia nervosa populations, which showed that observations of hyperactivity made by nurses, teachers or trained observers in day clinic or inpatient settings can be reliable and valid (Chen *et al.*, 2002; Fitzpatrick & Donovan, 1979; Stevens *et al.*, 1978). Accurate estimates of the activity levels in anorexia nervosa patients are clinically relevant, and given the complex nature of this phenomenon, should not be evaluated only with self-report, questionnaires or with interviews. Instead, to accurately monitor a patient's activity level during treatment, we should rely on the skills and observation of trained nurses.

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The impact of hyperactivity and leptin on recovery from anorexia nervosa

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4

Abstract

In Anorexia Nervosa (AN), hyperactivity is observed in about 80% of patients and has been associated with low leptin levels in the acute stage of AN and in anorexia animal models. To further understand the importance of this correlation in AN, we investigated the relationship between hypoleptinaemia and hyperactivity in AN patients longitudinally and assessed their predictive value for recovery.

Body weight, activity levels, and serum leptin levels were assessed in adolescents and adult AN patient groups at the start and during treatment, up to a year. In the adolescent group, initial leptin and activity levels were correlated. This negative correlation changes over time into a positive correlation with physiological recovery. Treatment outcome in both groups could be predicted by initial BMI and leptin levels but not by activity levels. No major relationship of activity with the course of recovery was detected, suggesting that in contrast to the acute stage of the disease, leptin and activity levels during the recovery process are dissociated.

Introduction

Anorexia nervosa (AN) is a complex eating disorder of unknown etiology, which primarily affects adolescent girls and young women (Hoek and Hoeken, 2003; Lucas et al., 1991; Lucas et al., 1999). It is characterized by aberrant patterns of eating behavior and weight regulation which result in weight loss and endocrine abnormalities such as amenorrhea, disturbances in attitude and perception about weight and shape, and an intense fear of gaining weight (American Psychiatric Association, 1994).

Other symptoms, such as perfectionism, obsessive-compulsive behavior, and social anxiety are observed in many but not all patients (Casper and Jabine, 1996; Piran et al., 1985). In addition, a proportion of AN patients (31–80 %, Hebebrand et al., 2003) displays abnormally high physical activity levels and overexercises. Almost all patients show a constant, agitated restlessness when they are emaciated but before they become lethargic in the final stages of starvation. The exact nature of hyperactivity in anorexia nervosa remains to be clarified. It appears that neurobiological factors and conscious attempts to burn calories in order to loose more weight coexist (Casper, 1998 2006).

Animal models mimicking AN weight loss and hyperactivity, such as the activity based anorexia (ABA) model (Routtenberg et al., 1967; Kas et al., 2003) or the semistarvation induced hyperactivity (SIH) model (Pirke, 1993; Exner et al., 2000) have been helpful in the search for a possible biological drive or changes in physiological parameters that trigger food restriction and hyperactivity. Studies using these preclinical models showed that anorectic rats (Kas et al., 2003) and mice (Gelegen et al., 2006) have reduced plasma leptin levels and that treating food restricted rats with leptin suppresses the development of hyperactivity (Hillebrand et al., 2005a; Exner et al., 2000).

Leptin is an adipocyte secreted hormone, product of the ob gene (Zhang et al., 1994), which plays a pivotal role in starvation situations with regard to energy homeostasis as well as other physiological processes, including reproductive functioning (Cioffi et al., 1996; Moschos et al., 2002). Plasma leptin levels rapidly decrease during weight loss (Ahima et al., 1996) and are extremely low in AN patients (Calandra et al., 2003). However, leptin levels in recently weight recovered patients have been found to be higher than in BMI-matched

controls, and are thought to contribute to difficulties with further weight restoration and maintenance (Mantzoros et al., 1997; Eckert et al., 1998; Holtkamp et al., 2004). Holtkamp et al. have shown that patients leptin levels are negatively correlated with motor restlessness scores (2003) and with physical activity in acutely ill AN patients (2006). Thus, pre-clinical and clinical observations suggest that hypoleptinemia is an important factor underlying excessive physical activity in AN. The meaning of these findings for recovery have however not been studied in detail to date.

The objective of this study was to investigate the relationship between hyperactivity, serum leptin levels, and recovery in AN patients.

Materials and Methods

Subjects

Patients were included in the study after they and/or their parents gave informed consent. The sample was recruited in two specialized eating disorder treatment centers (clinics), one for adolescents 12–17 years (the University Medical Center Utrecht [UMCU], 31 subjects) and one for adults older than 17 years (Rintveld, 30 subjects). The clinical research protocol for this prospectively designed, cohort follow-up study was approved by the UMCU Ethics Review Committee.

Inclusion criteria were: (1) AN diagnosed according to the DSM-IV (American Psychiatric Association 1994) on the basis of a structured interview using the Eating Disorders Examination (Cooper et al 1989); (2) comorbidity restricted to depression or anxiety disorders; (3) secondary amenorrhea ; (4) no use of steroid contraceptives; (5) no clinical history of concurrent illnesses. Sixty-one female patients were included, 24 of the AN purging type, 37 of the AN restricting type.

Study design

After initial psychiatric assessment, patients entered a structured treatment program aimed at restoration of the patient's weight, normalization of eating patterns, body image, anorectic

cognitions and family and social functioning. Weight gain was targeted at 0.5–1.0 kg/wk in accordance with clinical guidelines. Weight recovery was defined as a weight within the normal range for age (> SD -1.5 corresponding with a Body Mass Index [BMI] of approximately 19 kg/m^2 for adults) and target weight as the weight at which patients resumed a regular menstrual cycle, defined as three menstrual periods at three to five week intervals. Individual, group and family therapy techniques were used to change the patients' aberrant body perception and cognition.

Data was collected from patient acceptance into the treatment program, in the acute stage of the illness, until the end point, defined as recovery (return of regular menses), or up to one year after begin of treatment.

Body weight was measured once a week. The degree of underweight was calculated using BMI (kg/m^2) and BMI computed into z scores which describe the distance in SD from the mean BMI for that age (using a software program provided by the Netherlands Organization for Applied Scientific Research to account for differences in age) and to relate the data to Dutch population references (Van Buuren and Fredriks, 2001).

Blood samples for hormonal analysis were obtained by venepuncture at admission and every two weeks thereafter at 4 pm, up to the maximum study period of one year.

Activity measures

Physical activity levels were measured by trained nurses once a week. In an earlier study using actometer measurement as gold standard we established the reliability and validity of nurse observations of patient activity (Van Elburg et al., in press). Nurses were instructed to observe activity, defined as the amount of motor restlessness (inability to sit still or moving arms or legs while seated, walking through the ward without reason), abnormal motor activity, and excessive exercise, and to score their observations on a visual analogue scale (0-10).

Leptin assay

Plasma samples were stored at -80°C prior to determination. Leptin was measured using a sensitive RIA (Human-Leptin-RIA sensitive, Mediagnost, Tubingen, Germany), intra-assay CV of 5%, inter-assay CV of 7.6%.

Statistics

At the study endpoint, follow-up data was collected on the basis of which, patients were divided into three groups according to their recovery stage: no weight recovery (NWR), weight recovery without resumption of menses (WR) and weight and cycle recovery (WCR). Data are presented as the mean ± SD if distributed normally, otherwise as the median and range. Kruskal-Wallis tests were used if data were not normally distributed. In case data were normally distributed ANOVA was used. Linear multiple regression analysis (backward elimination) was also carried out to predict physical activity using BMI z scores and log 10 leptin levels as predictor variables. A p-value of < 0.05 was considered to be statistically significant.

To determine the associations of weight, leptin and physical activity levels with the rate of recovery as defined by the menstrual cycle at end point, univariate and multivariate Cox proportional hazards analyses were performed. This method of analysis estimates a linear regression model of parameters against the logarithm of the hazard (or instantaneous risk) of the menstrual cycle recovery during follow-up. Initial values of parameters were used as potential predictors. Parameters with univariate $p < 0.30$ were candidate predictors for the multivariate model; backward elimination of parameters (with $p < 0.05$ for inclusion) was used to determine the set of most predictive parameters. Because of potential differences in prognosis averages between the two treatment centers, the analysis was stratified by clinic. The analysis was first perfomed with initial values of weight, leptin and physical activity levels as predictors and subsequently with longitudinal values, using Cox regression with time-dependent covariates. A further analysis of longitudinal data focused on the evolvement of the association between leptin levels and activity levels during the course of treatment. A linear mixed model was used, predicting physical activity from log 10 leptin levels, follow-up time measured in weeks and final recovery status as predictor variables. A potential difference between the recovered and non-recovered group in the evolvement of the association between leptin levels and activity levels over time was tested by an interaction of (log 10 leptin)*week*group.

Statistical analysis was performed using SPSS for Windows (release 11.5, SPSS Inc., Chicago, IL) and S-plus software (MathSoft, Inc., Seattle, WA, version 2000).

Results

Mean age of the whole patient group was 18.2 ± 3.1 (SD) years, 15.9 ± 1.2 years for the adolescent group and 20.6 ± 2.9 years for the adult group. Average BMI at admission was 15.4 ± 1.3 kg/m^2 , which corresponds to a BMI z score of -3.8 ± - 1.6 (Table 1). Within the one year time frame of the study, 42 (69%) patients met the weight recovery objectives. Of them 24 (39% of the total, 57% of the weight recovered group) also recovered their menstrual cycles; 18 patients recovered in weight without the resumption of a menstrual cycle. Nineteen patients (31%) remained at a low weight throughout the year.

There were no significant differences among the three outcome groups in initial body weight, duration of illness (defined by duration of amenorrhea), duration of study participation, type of AN (restrictive or purging type), plasma leptin levels, nurse ratings of physical activity levels, amount of prescribed neuroleptics, or SSRI's (Table 1). 14 patients used olanzapine, 7 in each group (clinic) with dosages ranging from 5 to 15 mg/day, average dose 7.5 mg in both clinics. A difference in age was observed. The group that recovered in weight and resumed a menstrual cycle was significantly older than the non-recovered group, which result also corresponds with the clinic where they were treated. Rates of recovering weight and menstrual cycle differed in the two clinics: 16% of the patients in the adolescent group recovered versus 63% of the patients in the adult group.

Table 1. Demographics (mean + SD) of 61 young women diagnosed with anorexia nervosa. Data are presented for the entire group, and separately for women presenting with no weight recovery (NWR), with weight recovery (WR) only and with weight + cycle recovery (WCR) over a period of maximum 12 months.

Variables	Total group	No weight recovery, Amenorrhea (NWR) (n=61)	Weight recovery, Amenorrhea (WR) (n = 19)	Weight + cycle recovery (WCR) (n = 18) (n = 24)
Age (years)	18.2 ± 3.1	17.3 ± 2.2	16.9 ± 3.2	19.8± 3.0* vs. NWR+ WR
Weeks in study	33.5 ± 11.4	32.8 ± 11.6	36.9 ± 14.0	31.6 ± 8.8
Bodyweight, initial z scores	-3.8 ± 1.6	-4.2 ± 1.9	-3.2 ± 1.5	-3.8 ± 1.2
Bodyweight end of study	-1.3 ± 1.1	-2.1 ± 0.8* vs. WCR+ WR	-0.9 ± 0.5	-0.9 ± 0.6
Amenorrhea (months)	24.4 ± 22.8	20.5 ± 13.5	30.0 ± 30.5	23.9 ± 23.6
Initial Leptin (µg/l)	2.1 (0.5-13.3)	2.9 (0.7-8.6)	1.5 0(.5-4.6)	2.4 (0.7-13.3)
AN Restrictive type (%)	69	79	72	58
Neuroleptics (%)	57	47	55	58
SSRIs (%)	33	26	22	50
Initial Activity score	52.5 ± 26.9	49.1± 27.9	56.2 ± 24.9	52.4 ± 28.8

*$p < 0.01$

For the group as a whole, initial leptin levels did not correlate with nurse ratings of activity, the scatter plot showing a U-shape (p=0.082). After stratification by clinic and adjusted for BMI z sore, the adolescent patient group showed a linear association between activity levels and log 10 serum leptin levels (partial correlation: -0.40, p=0.027), whereas the adult group shows the U-shaped scatter plot, p=0.12 (Figure 1).

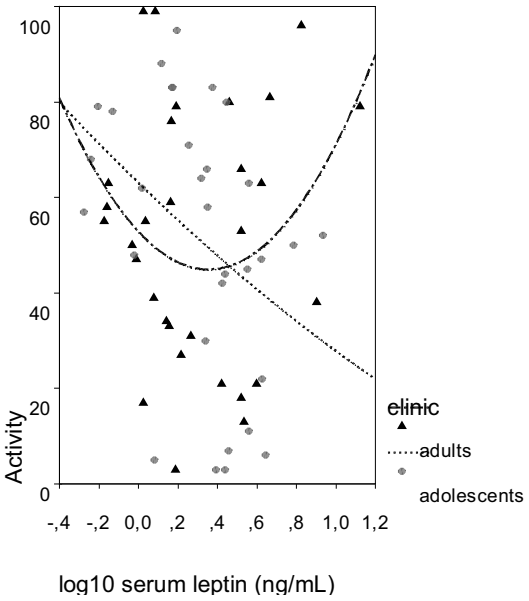

Figure 1. Scatter plot of initial log 10 serum leptin levels versus observed levels of levels of activity in two groups of patients with AN. Activity is defined as the amount of motor restlessness, abnormal motor activity and excessive exercise, as observed by nurses. p=0.027 for the adolescent group (N=31), p=0.12 for the adult group (N=30).

Next, we analyzed the predictive value of initial BMI z score, activity and plasma leptin levels on complete (weight and cycle) recovery. In a univariate analysis, initial leptin levels and initial BMI z scores predicted a favourable outcome, whereas initial activity levels showed no correlation. Multivariate analysis showed that leptin was the sole significant variable (Table 2).

Parameter	Univariate *p* Value	Multivariate *p* Value	Hazard Ratio* (95% CI)
BMI z score	0.0014	0.15	
Leptin Level	< 0.001	< 0.001	1.97 (1.31 – 2.97)
Activity Level	0. 84	0.96	

Table 2. Association of initial BMI z scores, leptin, and activity levels with time to recovery of menstrual cycle.

*The change in hazard when the parameter is increased by one standard deviation.

Cox regression stratified by treatment center

Analysis of the longitudinal data using a Cox model with time-dependent covariates showed leptin and BMI z score as main influences on recovery of menstrual cycle. Adding activity did not improve prediction in the model (Table 3).

Parameter	Univariate *p* Value	Multivariate *p* Value	Hazard Ratio* (95% CI)
BMI z score	< 0.001	.003	2.1 (1.3 - 3.3)
Leptin	< 0.001	.022	1.12 (1.02 - 1.24)
Activity	0.9		

Table 3. Time-dependent analysis of the association of BMI z scores, leptin and activity levels with recovery of menstrual cycle

* Hazard ratio for standardised parameters, with 95% confidence interval. The hazard ratio represents the change in hazard when the parameter is increased by one standard deviation.

Cox regression stratified by clinic.

The linear mixed model analysis showed that the recovered (WCR) and non-recovered patients (WR and NWR) differed significantly in the way the relationship between leptin

and physical activity changed during the treatment period (P = 0.016, test for interaction): the correlation between leptin and physical activity remained the same during treatment (r = -0.28) in the NWR and WR groups, whereas in the WCR group it changed from an initial value of r = -0.22 at 16 weeks to r = +0.58 at 52 weeks.

Discussion

In this longitudinal study we measured body weight, plasma leptin levels, and activity levels in a clinical sample of AN patients during recovery.

We replicated the finding from animal and human studies (Kas et al., 2003, Holtkamp et al., 2003, 2006) that initial log 10 leptin levels and activity levels in the acute stage of the illness in a young patient population are correlated, and found a linear correlation in our adolescent age group. In our adult group we found a U shaped correlation between activity and plasma leptin levels, which suggests that hyperactivity is age-related. Over time however, physical activity did not predict outcome and no major relationship between physical activity levels and the course of recovery could be detected for neither the adolescent nor the adult group.

Contrary to Holtkamp (2003) we rated activity during treatment and observed by nurses instead of relying on anamnestic reports of physical activity in the months before admission or during the acute stage of the illness. In an earlier study (Van Elburg et al., in press) we showed that nurses can reliably rate physical activity levels of anorexia nervosa patients. Measurements of a patient's physical activity levels by nurses correlate with activity scores from actometers, the gold standard reference. Patient self-reports did not correlate with the actometer scores. In clinical practice a variety of methods has been used to evaluate hyperactivity, but based on our previous findings conclusions drawn from studies using self-report or expert report not based on observation should be considered only with caution.
Once patients were divided into different outcome groups at the end of the study period differences in levels of physical activity and leptin were found, even though initial levels were not significantly different. Initial plasma leptin levels for all groups were clearly below normal

values. Only the WCR-group reached average levels in the normal range at the end of the study period. WR patients clearly responded to treatment and recovered in weight, however, their subnormal leptin levels most probably did not permit them to recover at the level of the gonadal axis.

Several studies (Wang et al., 2006; Haupt et al., 2005) have discussed the relationship between leptin and the use of atypical antipsychotics such as olanzapine, sofar only in normal weight schizophrenic or psychotic patients. The results show that olanzapine in the first weeks of treatment may cause a surge in circulating leptin (Wang et al.) but that elevated plasma leptin levels in chronically treated patients with schizophrenia are strongly predicted by adiposity, similar to untreated healthy individuals (Haupt et al).

In a previous study (Hillebrand, 2005b), we demonstrated that olanzapine reduces activity levels in AN patients without affecting body weight and plasma leptin levels. In our current study, 57% of patients used antipsychotics (neuroleptics), which might have influenced the relationship between leptin and activity scores. However, Cox regression analysis with medication as predictor showed no correlation (univariate: neuroleptics: $p = 0.6$, SSRI's: $p = 0.3$). Holtkamp et al. (2003; 2006) unfortunately do not report use of neuroleptics in their study populations.

In summary, our study replicates earlier findings in both rodents and humans that hypoleptinaemia and high physical activity levels are related in acutely ill AN patients of a young age, but we did not find the same relationship in an older patient group. The negative correlation between leptin and activity that exists in the acute phase of the illness changes over the course of 16 weeks to become a positive correlation in the second half of the treatment period, as a result of recovery. In the non recovered patients the negative correlation continues.

Furthermore, our data indicate that plasma leptin level is a good predictor of recovery in AN, however, we could not detect any major relationship between physical activity levels and the course of recovery. The latter indicates that leptin and physical activity levels during the recovery process are, in contrast to the acute stage of the disease, dissociated. Further detailed assessment of this relationship is required to elaborate on these findings and their implications for clinical practice.

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Olanzapine reduces physical activity in rats exposed to activity-based anorexia: implications for treatment of anorexia nervosa

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Abstract

Background: Anorexia nervosa (AN) is a severe psychiatric disorder with a high mortality rate. Patients suffering from AN often show extreme hypophagia and frequently display excessive physical activity. Animal models of AN might contribute to the understanding of this illness and subsequently improve treatment. Activity-based anorexia (ABA) is considered an animal model of AN and mimics food restriction and hyperactivity in rats. Treatment with olanzapine (Zyprexa®), an atypical antipsychotic with limited extrapyrimidal effects and associated with body weight gain, was evaluated in the ABA model and AN patients.

Methods: Rats exposed to the ABA model were chronically infused with olanzapine (7.5 mg/kg/day) and development of ABA was studied. Furthermore, the effects of olanzapine treatment (5 mg) in AN patients were studied in a small clinical study.

Results: Olanzapine treatment in rats exposed to the ABA model significantly reduced running wheel activity. Olanzapine also reduced starvation-induced hypothermia and decreased HPA axis activation in ABA rats. In addition, olanzapine treatment reduced activity levels of AN patients as compared to untreated AN patients in the clinical study.

Conclusions: Olanzapine treatment is effective in reducing physical activity levels in both rats and AN patients (in a small open label study).

Introduction

Anorexia nervosa (AN) is a psychiatric disorder often characterized by extreme hypophagia, obsessive fears of being fat and hyperactivity (Casper et al 1991; Davis 1997; Kron et al 1978). When compared to other psychiatric disorders, AN has the highest mortality rate (Sullivan 1995).

Some studies show that serotonin (5-hydroxytryptamine, 5-HT) signaling is altered in ill AN patients as well as in recovered AN patients (Brewerton et al 1996). For example, AN patients show decreased cerebrospinal fluid (csf) levels of 5-HT and 5-hydroxyindole acetic acid (5-HIAA) during disease and increased levels of 5-HIAA when recovered (Kaye et al 1991). Studies on the use of serotonin reuptake inhibitors (SSRIs) in malnourished AN patients showed no benefits of treatment (Kaye et al 1998). However, SSRIs seem to be effective in preventing relapse in recovered AN patients (Kaye et al 2001). Genetic association studies showed possible implications of polymorphisms in the 5-HT_{2A} receptor, 5-HT_{2C} receptor and serotonin transporter in AN (Collier et al 1997; Di Bella et al 2000; Hu et al 2003; Nacmias et al 1999; Westberg et al 2002), although other studies could not confirm these findings (Ando et al 2001; Gorwood et al 2002; Hinney et al 1997; Nishiguchi et al 2001).

Trials on antipsychotic treatment in AN patients have been performed previously, but only to a limited extent (Hoffman et al 1993; Johnson et al 1983; Pederson et al 2003). Controlled studies on chlorpromazine (Dally et al 1966) and pimozide (Vandereycken et al 1982) showed increased weight gain during treatment and a more positive attitude of AN patients towards treatment, whereas the atypical antipsychotic sulpiride did not significantly influence body weight gain and eating- and body attitudes in AN patients (Vandereycken 1984).

Olanzapine (Zyprexa®) is an atypical antipsychotic with a broad pharmacological profile. This thienobenzodiazepine compound has high affinity for 5-$HT_{2A/2C}$ receptors, H_1 receptors and α_1 receptors and moderate affinity for D_1-D_4 receptors (Bymaster et al 1997; Moore 1999; Bymaster et al 1996; Schotte et al 1996). Olanzapine treatment in humans causes limited extrapyrimidal effects and has been associated with body weight gain (Allison et al 2001). In rats, acute injections of olanzapine increase feeding behavior (Thornton-Jones et al 2002)

and reduce locomotor activity (Prinssen et al 2000). The effects of olanzapine on body weight gain, food intake and locomotion suggest that olanzapine might influence pathophysiological processes in anorexia. Yet data on chronic (instead of acute) administration of olanzapine in rodents is rare (Pouzet et al 2003), although this is required for clinical comparisons (Kapur et al 2003).

The activity-based anorexia (ABA) model is used to study anorectic behavior in rodents and serves as an animal model of AN (Routtenberg et al 1967). In the ABA model, scheduled feeding in combination with voluntary access to running wheels, leads to a paradoxically increase in running wheel activity (RWA) and decrease in food intake (as compared to food-restricted controls) (Routtenberg et al 1967). This results in activation of the hypothalamus-pituitary-adrenal (HPA) axis and substantial body weight loss.

In the present study we investigated whether chronic olanzapine treatment influences the development of ABA in rats. Furthermore, we examined the effect of olanzapine treatment in hyperactive AN patients. A few (uncontrolled) studies already reported beneficial effects of olanzapine treatment on food intake and anxiety of AN patients, but no study reported effects of olanzapine treatment on activity levels before (Boachie et al 2003; Malina et al 2003; Mehler et al 2001; Powers et al 2002).

Material and Methods

Rats

Female outbred Wistar WU rats (n=30) (Harlan, Horst, The Netherlands) weighing 160 g upon arrival were individually housed in a temperature and humidity controlled room (21±2°C) under a 12:12 hr light:dark cycle (lights off at ±12:00 hr). The University of Utrecht ethical committee on use and care of animals approved all described procedures.

Drugs

For the animal studies olanzapine was kindly provided by Eli Lilly (Indianapolis, Indiana, USA). Olanzapine was dissolved in a minimum quantity of acetic acid, made up to volume

with sterile isotonic saline and adjusted to pH 6 with 5 M NaOH. Olanzapine was continuously infused (subcutaneously) during one week at a concentration of 7.5 mg/kg/day using osmotic minipumps (Alzet, model 2001, DURECT Corporation, Cupertino, California, USA) (Kapur et al 2003). In the human study, AN patients received Zyprexa® (5 mg) tablets (Eli Lilly).

Surgical procedures animal studies

Experiment 1 and 2: Transmitters (TA10TA-F40 Data Sciences International, St. Paul, Minnesota, USA) were placed in the abdominal cavity under fentanyl/fluanisone (Hypnorm®, Janssen Pharmaceutica, Beerse, Belgium, 0.1 ml/100 g im) and midazolam (Dormicum®, Hoffman-LaRoche, Mijdrecht, The Netherlands, 0.05 ml/100 g ip) anesthesia. After surgery, rats were treated with buprenorphin (Temgesic®, Schering-Plough, Maarssen, The Netherlands, 0.05 ml/100 g sc) and saline (1 ml sc) and were allowed to recover for two weeks.

For chronic infusions, osmotic minipumps were filled with olanzapine or vehicle and were activated by overnight incubation at 37°C. The next day (day -1) pumps were positioned subcutaneously into the flank of the rats under Hypnorm® anesthesia. After surgery, rats were treated with Temgesic® and saline as indicated above.

Experimental set-up animal studies

Experiment 1: Transmitters were implanted one week after arrival of the rats. After two weeks of recovery (day –10), the rats (n=14, synchronized for estrous cycle) were placed in cages with running wheels for adaptation to the running wheel. During this ten-day period, food and water were available ad libitum. RWA was continuously registered using a Cage Registration Program (Dept. Biomedical Engineering, UMC Utrecht, The Netherlands). At the end of day –2, transmitters were activated for baseline recordings of body temperature. At the end of day –1, rats were divided into two groups, matched for body weight (vehicle: 214.7±2.5 g, olanzapine: 215.0±2.7 g, n.s.) and baseline RWA. Baseline RWA was determined as average RWA during four days prior to the start of infusion (day -4 to day –1) (vehicle: 5281±1409 revolutions, olanzapine: 5694±1496 revolutions, n.s.). Osmotic minipumps containing olanzapine or vehicle were implanted as indicated above. Immediately after surgery, food was removed (=start day 0). The next days (day 1-6) rats had one hr access to food (first hr of

the dark phase), while water was available ad libitum. Body weight and food intake were measured daily. At the end of day 6 (end light phase) rats were decapitated and trunk blood was collected into lithium-heparin (Sarstedt, Nümbrecht, Germany) containing tubes with 83 μmol EDTA and 1 mg aprotonin. Tubes were kept on ice until centrifugation (20 minutes at 3000 rpm 4°C), subsequently plasma was stored at –20°C. Retroperitoneal white adipose tissue (rWAT), interscapular brown adipose tissue (iBAT) and adrenals were collected and weighed.

Experiment 2: This experiment was performed similar to experiment 1, the only difference being that rats had ad libitum access to food during the whole experiment. Sixteen rats were divided into two groups, matched for body weight (vehicle: 236.2±4.7 g, olanzapine: 231.2±5.9 g, n.s.) and baseline RWA (vehicle: 6686±1124 revolutions, olanzapine: 6514±1590 revolutions, n.s.).

Patients

In the open label trial AN patients were studied in a specialized treatment setting. At their entrance to the hospital, activity levels of AN patients were scored by trained nurses on a scale from 0 to 100 (score 0= inactive, score 100=extremely active), which has recently been validated by using Actiwatches (manuscript in preparation).

From a cohort of 22 AN patients, 13 patients displayed activity scores higher than >50 at their entrance to the hospital and were entitled hyperactive (13/22= 59%) and included in this study. Of these 13 female AN patients, 5 patients received olanzapine treatment (5 mg) (age:17.1±1.1 yr), while 8 patients received no medication (age:17.0±0.4 yr). Patients were attributed to the olanzapine treatment group because of their anxious behavior towards eating and body weight gain. All AN patients were free from other forms of pharmacotherapy. Additional treatment was in general the same for all patients and was aimed at body weight gain (0.75 kg/week) followed by further normalizing of cognition and body image and treatment of possible co-morbid problems. Both groups contained in-patients as well as daytreatment-patients (who visited the hospital twice weekly). Every week activity levels were scored. Body weight (z-scores to control for age) was measured and once in two weeks blood was collected. All AN patients and parents (in case of minors) gave their informed consent for participation

in a clinical study, which was approved by the Ethical Committee of the University Medical Center of Utrecht.

Radioimmunoassay

In rats, plasma levels of corticosterone, adrenocorticotropic hormone (ACTH) and leptin were measured by radioimmunoassays (RIA). Plasma levels of corticosterone were measured using a commercially available rat corticosterone RIA kit (ICN Biochemicals, Costa Mesa, California, USA). Plasma ACTH was measured using a specific rabbit antiserum directed to the midportion of ACTH, which was kindly provided by Dr. G.B. Makara (Budapest, Hungary). Synthetic human $ACTH_{(1\text{-}39)}$ (Peninsula Laboratories, Belmont, California, USA) was labeled with ^{125}I and used as a tracer (Nijsen et al 2000). Plasma levels of leptin were measured using a commercially available rat leptin RIA kit, according to the manufacturer's protocol (Linco Research, St. Charles Missouri USA). In patients, plasma levels of leptin were analyzed by the DLR-Institute of Aerospace Medicine, Space Physiology in Cologne, Germany using a sensitive human leptin RIA assay (Mediagnost Reutlingen, Germany).

Data analysis

Data are presented as mean ± standard error. For all measurements (in animal studies and the human study), baseline levels were not significantly different between olanzapine-treated and vehicle-treated (no-medication in the human study respectively) groups.

Basal body temperature of rats was defined as mean body temperature during 30 minutes of inactivity in the early light phase measured by telemetric devices. RWA, body weight, food intake and basal body temperature data were first attributed to repeated measures analysis, using Huynh Feldt correction for Mauchlys sphericity effects, followed by t-test or Mann Whitey U test when appropriate. rWAT, iBAT, plasma leptin levels and HPA axis activation were analyzed by independent t-tests.

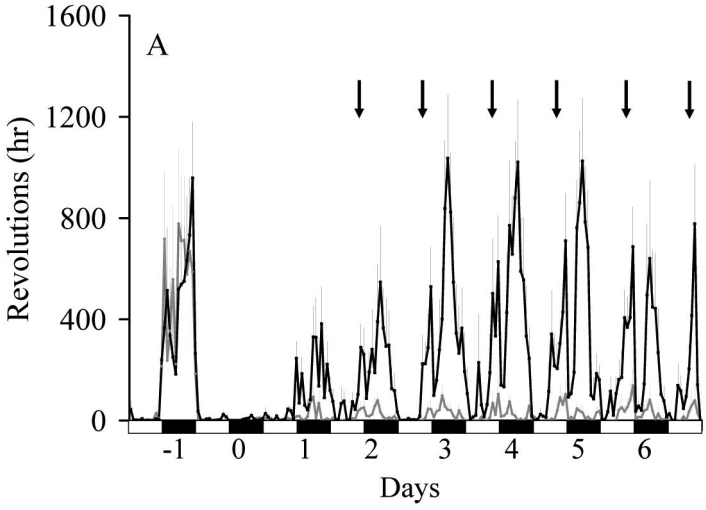
Weekly activity scores were averaged to monthly scores (to diminish variation within the same individual) and were attributed to repeated measures analysis using Huynh Feldt correction for Mauchlys sphericity effects, followed by t-tests. Differences in body weight (z-scores) and plasma leptin levels between the two patient groups were determined at the onset

of the study and following three months of treatment by t-tests. Differences were considered significant at $p<0.05$. In two AN patients, olanzapine treatment lasted for two months instead of three months. Consequently, activity, body weight and plasma leptin levels in month three were analyzed in three (instead of five) olanzapine-treated patients.

Results

Olanzapine treatment in activity-based anorexia

Olanzapine treatment significantly decreased running wheel activity (RWA) in ABA rats (day: $F(6,66)=8.55$; $p=0.001$, day x treatment: $F(6,66)=6.75$; $p=0.001$). Vehicle-treated ABA rats increased RWA following the start of scheduled feeding. Not only daily revolutions increased, the distribution of activity changed too; a substantial part of total activity occurred prior to the feeding period (= food anticipatory activity, FAA). FAA is one of the key phenomena occurring in ABA and (in this experimental setup) took place in the light phase. Olanzapine-treated ABA rats reduced RWA in the dark phase (day: $F(6,66)=6.02$; $p=0.002$, day x treatment: $F(6,66)=5.23$; $p=0.004$) and showed decreased FAA (day: $F(6,66)=7.44$; $p=0.002$), day x treatment: $F(6,66)=4.68$; $p=0.02$) (Figure 1).



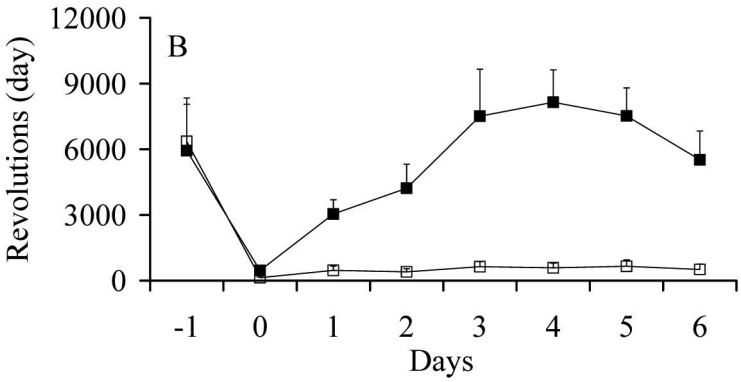


Figure 1. Running wheel activity in activity-based anorexia rats.

A. Running wheel activity (RWA) per hour in activity-based anorexia (ABA) rats (day -1 (baseline) - day 6) following vehicle (black) or olanzapine (grey) treatment. Arrows indicate food anticipatory activity (FAA). B. Total RWA per day (day -1 (baseline) – day 6) of ABA rats following vehicle (closed squares) or olanzapine (open squares) treatment. *=p<0.05, Mann Whitney U test.

One hr food intake was not significantly affected by olanzapine treatment over time (day: $F(5,60)=23.62$; $p=0.001$, day x treatment: $F(5,60)=0.84$; n.s.). Olanzapine treatment tended to reduce body weight loss (day: $F(6,72)=129.45$; $p=0.001$, day x treatment: $F(6,72)=3.64$; n.s.) which was consistent with a trend towards a higher rWAT weight ($t(12)=-0.92$; n.s.) (Table 1). However, plasma leptin levels were below detection limits in vehicle-treated as well as olanzapine-treated ABA rats.

Treatment	Food(g)	Body weight (%)	rWAT (mg)	iBAT (mg)
Vehicle	39.7±2.4	81.7±1.9	278.6±97.2	98.9±6.3
Olanzapine	39.5±1.4	84.3±0.4	404.0±80.6	147.0±15.1*

Table 1. Cumulative (day 1-day 6) food intake, relative body weight (% of day -1), retroperitoneal white adipose tissue (rWAT) weight and interscapular brown adipose tissue weight (iBAT) (all measured at the end of day 6) were analyzed in activity-based anorexia (ABA) rats treated with vehicle or olanzapine. *=p<0.05, t-test.

Basal body temperature in ABA rats was higher following olanzapine treatment as compared to vehicle treatment (day: F(6,60)=18.02; p=0.001, day x treatment: F(6,60)=5.43; p=0.02) (Figure 2) and iBAT weight was significantly increased (t(12)=-2.94; p=0.02) in olanzapine-treated ABA rats.

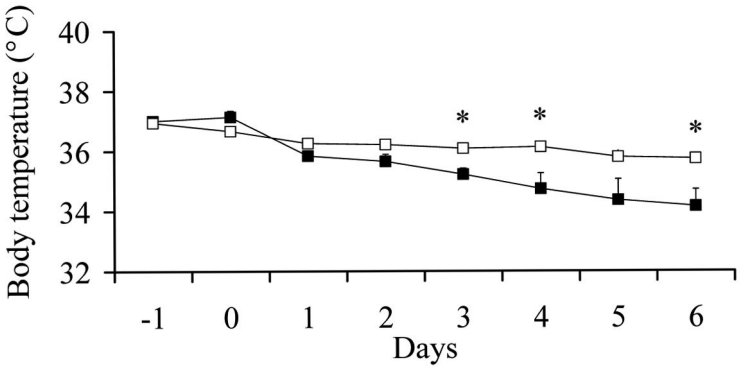


Figure 2. Basal body temperature in activity-based anorexia rats.

Basal body temperature of activity-based anorexia (ABA) rats (day -1 (baseline) – day 6) following vehicle (closed squares) or olanzapine (open squares) treatment. *= p<0.05, t-test.

HPA axis activation was decreased following olanzapine treatment. ACTH plasma levels were significantly reduced (t(12)=3.46; p=0.005), while corticosterone plasma levels (t(12)=0.68; n.s.) and adrenal weights (t(12)=1.08; n.s.) tended to be decreased (Table 2).

Treatment	ACTH (pg/ml)	Corticosterone (µg/dl)	Adrenal weight (mg)
Vehicle	167.1±14.4	41.1±7.4	71.6±3.9
Olanzapine	114.7±4.5 *	34.7±5.7	65.6±4.0

Table 2. Adrenocorticotropic hormone (ACTH) plasma levels, corticosterone plasma levels and adrenal weight (all analyzed at the end of day 6) were measured in activity-based anorexia (ABA) rats treated with vehicle or olanzapine. *=p<0.05, t-test.

Thus, treating ABA rats with 7.5 mg/kg olanzapine per day reduced RWA, tended to decrease body weight loss without significantly affecting food intake, decreased HPA axis activation, and reduced starvation-induced hypothermia. To investigate whether the effects of olanzapine described above were specific for the ABA model, the experiment was repeated with ad libitum fed running rats.

Olanzapine treatment in ad libitum fed running rats

The reduction in RWA described above was not specific for ABA rats. Olanzapine treatment (7.5 mg/kg/day) also decreased total RWA (day: $F(6,84)=5.36$; $p=0.02$, day x treatment: $F(6,84)=4.89$, $p=0.02$) by reducing dark phase RWA (day: $F(6,84)=5.38$; $p=0.01$, day x treatment: $F(6,84)=4.86$, $p=0.02$) in ad libitum fed running rats (Figure 3). Light phase RWA of ad libitum fed rats was negligible.

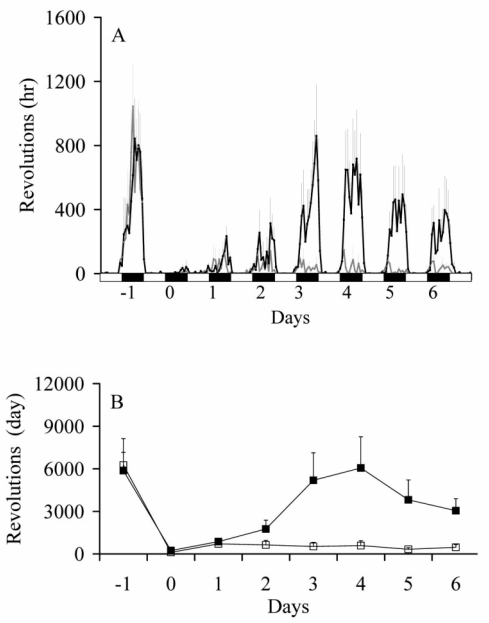


Figure 3. Running wheel activity in ad libitum fed rats.

A. Running wheel activity (RWA) per hour in ad libitum fed rats (day -1 (baseline) - day 6) following vehicle (black) or olanzapine (grey) treatment.

B. Total RWA (day -1 (baseline) - day 6) of ad libitum fed rats following vehicle (closed squares) or olanzapine (open squares) treatment. *=$p<0.05$, Mann Whitney U test.

Olanzapine treatment significantly increased food intake (day: F(6,84)=27.67; p=0.001, day x treatment: F(6,84)=3.84, p=0.02) and relative body weight (day: F(6,84)=75.08; p=0.001, day x treatment: F(6,84)=12.51, p=0.001) in ad libitum fed rats. rWAT mass tended to be increased in olanzapine-treated ad libitum fed running rats (t(14)=-0.91; n.s.), whereas plasma leptin levels were significantly increased (t(14)=-3.71 p=0.002) (Table 3).

Treatment	Food (g)	Body weight (%)	rWAT (mg)	iBAT (mg)	Leptin (ng/ml)
Vehicle	141.0±3.6	105.9±1.3	2646.7±300.8	230.1±20.6	2.48±0.45
Olanzapine	153.2±4.6 *	109.4±0.8 *	3023.3±288.3	261.1±38.1	5.14±0.56 *

Table 3. Cumulative (day 1-day 6) food intake, relative body weight (% of day -1), retroperitoneal white adipose tissue (rWAT) weight, interscapular brown adipose tissue weight (iBAT) and plasma leptin levels (all measured at the end of day 6) were analyzed in ad libitum fed rats treated with vehicle or olanzapine. *=p<0.05, t-test.

Basal body temperature as well as iBAT weight were not significantly affected (day: F(6,84)=2.44; n.s., day x treatment: F(6,84)=1.02; n.s.), (t(13)=-0.68; n.s.) (Figure 4). Olanzapine treatment did not significantly influence plasma ACTH levels (t(14)=0.48; n.s.) and plasma corticosterone levels (t(14)=1.27, n.s.), but decreased adrenal weight (t(14)=2.94, p=0.003) in ad libitum fed running rats (Table 4).

Treatment	ACTH (pg/ml)	Corticosterone (µg/dl)	Adrenal weight (mg)
Vehicle	132.6±11.2	32.2±2.7	70.2±2.4
Olanzapine	124.9±11.4	26.7±3.4	58.6±3.1 *

Table 4. Adrenocorticotropic hormone (ACTH) plasma levels, corticosterone plasma levels and adrenal weight (all analyzed at the end of day 6) were measured in ad libitum fed rats treated with vehicle or olanzapine. *=p<0.05, t-test.

Olanzapine treatment in anorexia nervosa patients

Initial levels of physical activity were not significantly different between olanzapine-treated AN patients and AN patients without medication (olanzapine treatment: 61.6±8.8, no treatment: 66.3.±4.6) (t(11)=0.49; n.s.). Olanzapine treatment (5 mg per day) significantly affected activity levels of AN patients during treatment (month: F(3,27)=5.06; p=0.01, month x treatment F(3,27)=4.57; p=0.01). Activity levels were significantly decreased following 2 and 3 months of olanzapine treatment (Figure 5).

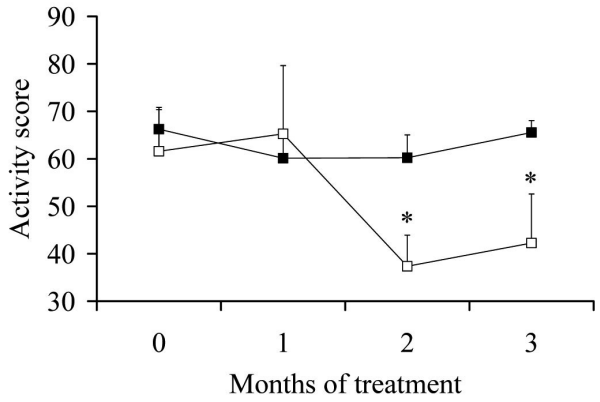


Figure 5. Effects of olanzapine treatment on hyperactivity in anorexia nervosa patients.
Activity scores of anorexia nervosa patients with olanzapine treatment (n=5, open squares) or without treatment (n=8, closed squares). Activity was scored on a 0-100 scale by trained nurses. Note that at month 3, only 3 patients (instead of 5) received olanzapine treatment. *= $p<0.05$, t-test.

Body weight of olanzapine-treated AN patients (z-score:-4.51±1.2) was not significantly different from body weight of AN patients without medication (z-score: -2.54±0.25) at the onset of the study (t(11)=1.98; n.s.). Following three months of treatment, body weight increased in both groups, but was not significantly different in olanzapine-treated AN patients (z-score: -2.93±0.36) and AN patients without medication (z-score: -1.79±0.32) (t(9)=2.02; n.s.). Plasma leptin levels of olanzapine-treated AN patients (plasma leptin: 1.45±0.55 ng/ml) were not significantly different from plasma leptin levels of AN patients without medication (plasma leptin: 3.80±1.47 ng/ml) at the onset of the study (t(11)=1.21; n.s.). Plasma leptin

levels significantly increased following three months of treatment in both groups, but were not significantly different between olanzapine-treated AN patients (plasma leptin: 3.72±0.38 ng/ml) and AN patients without medication (plasma leptin: 4.53 ± 1.32 ng/ml) after three months of treatment ($t(9)=0.36$; n.s.).

Discussion

In this study we showed that olanzapine reduces RWA in the ABA model and also reduces physical activity in AN patients. As far as we know this is the first study that describes the efficacy of olanzapine to reduce hyperactivity in AN.

Three important parameters of the ABA model are; increased RWA, decreased food intake and HPA axis activation. RWA was strongly decreased by olanzapine treatment in restricted and ad libitum fed rats with running wheels. Not only dark phase activity, but also light phase activity (including food-anticipatory activity, FAA) of ABA rats was reduced. Former studies showed that once exposed to scheduled feeding in the ABA model, rats decrease food intake as compared to restricted rats without running wheels (Routtenberg et al 1967). Thus, rats in the ABA model can be called anorectic. Olanzapine treatment did not influence one hr food intake in ABA rats, but increased food intake and body weight gain in ad libitum fed rats. Hence, it appears that the effects of chronic olanzapine treatment on food intake and body weight are different in ad libitum fed rats and ABA rats (although not investigated in the same experiment), whereas the effect on RWA is independent of energy status. The absence of an effect of olanzapine treatment on food intake in food-restricted rats is unclear. It has been reported before that olanzapine treatment enhances ingestive behavior in food-deprived rats (Thornton-Jones et al 2002). However, it is possible that the one hr feeding period in our experiment was too short to observe an orexigenic effect of olanzapine.

Previously it was shown that peripheral leptin treatment reduced hyperactivity of rats in the semi starvation-induced hyperactivity model (Exner et al 2000) and it was proposed that decreases in leptin signaling might trigger hyperactivity in rats, as well as in AN patients (Hebebrand et al 1997). In the present study, plasma leptin levels were undetectable in both

vehicle-treated and olanzapine-treated ABA rats. Thus, we showed that hyperactivity can be reduced in ABA rats, without restoring plasma leptin levels. This suggests that besides decreased plasma leptin levels, other factors (possibly 5-HT) may trigger development of hyperactivity. Our preliminary results from the human study also indicate that reduction of hyperactivity by olanzapine treatment is not significantly related to increases in plasma leptin levels, although absence of such an association might be due to the small sample size of the present study.

Hyperactivity in combination with food restriction results in rapid exhaustion of energy stores in ABA rats. After a few days of exposure to the ABA model, energy stores are depleted and body temperature can not be maintained (Kas et al 2003). Here we show that olanzapine treatment partly prevented the drop in body temperature. Basal body temperature of ad libitum fed rats was not altered following olanzapine treatment, which is in contrast with another study reporting dose-dependent hypothermia following olanzapine treatment (Oerther et al 2000).

As a result of restricted feeding and wheel running, the HPA axis is considerably activated in ABA rats (Burden et al 1993). In this study we showed that olanzapine treatment decreased ACTH levels and tended to decrease corticosterone levels and adrenal weight in ABA rats, thereby reducing stress levels in ABA rats. The absence of a significant effect on corticosterone levels might be explained by the time of measurement; blood plasma was collected during the circadian peak of the corticosterone rhythm.

This study is one of the first reporting chronic infusions of olanzapine in rats. At first glance, the dose used might appear extraordinary high and not in relation to the clinically effective dose. However, recently it was reported that chronic administration of olanzapine (by osmotic minipumps) requires infusion concentrations at least 5 times higher (7.5 mg/kg sc) than the optimal single dose (1-2 mg/kg sc) to achieve clinically comparable D_2 receptor occupancy (Kapur et al 2003). The difference in the olanzapine dose used in rats and humans, is attributed to the fast biotransformation of olanzapine in rats ($T_{1/2}$=2.5 hrs) as compared to humans ($T_{1/2}$ =21-53 hrs) (Aravagiri et al 1997; Kapur et al 2003). As a result, the 7.5 mg/kg dose of rats results in clinically comparable D_2 receptor occupancy, but would be supra-therapeutic in (AN) patients.

In the small open label study that we performed here, AN patients treated with olanzapine showed a significant reduction of hyperactivity, whereas activity levels of olanzapine-free patients did not change. Reducing activity levels in AN patients that are hyperactive during admission can be crucial for further therapeutic outcome. In the present study, nurses were not blind to the treatment condition, when assessing activity, which might be a bias in the results. Since AN patients were treated with olanzapine if they showed anxious behavior towards eating and body weight gain, it is possible that the reduction in activity levels is an indirect result of changes in anxious behavior, as reported earlier (Boachie et al 2003; Malina et al 2003). This might suggest that AN patients attempt to alleviate anxiety through exercising. Recently, Klein and co-workers postulated that AN patients, which showed dependence on exercise, also showed high scores on the Beck Anxiety Inventory (Klein et al 2004).

Only a few studies on olanzapine treatment in AN patients have been described before. Olanzapine increased body weight gain, reduced agitation and anxiety, increased ability or desire to eat and decreased obsessive thoughts concerning body image (Boachie et al 2003; Malina et al 2003; Mehler et al 2001; Powers et al 2002). These results combined with our data (showing that olanzapine reduced physical activity) can not exclude that olanzapine influences behavior in rats and patients by sedation. Although anticipated, body weight gain and plasma leptin levels of AN patients were not significantly affected by olanzapine treatment. This might be explained by the relative short time of medication and by the small sample size. For now, it appears that the reduction in activity is not related to changes in plasma leptin levels, similar to the results from the ABA model. However, future controlled studies using a larger sample size, should further explore the role of changes in plasma leptin levels in olanzapine-induced reduction of hyperactivity, since a trend towards a stronger increase in plasma leptin levels in olanzapine-treated patients was present.

Olanzapine has highest affinity for 5-$HT_{2A/2C}$ receptors. It has been widely described that 5-HT pathways play an important role in energy balance. Increased levels of 5-HT or direct activation 5-$HT_{2A/2C}$ receptors reduces food intake (Clifton et al 2000; Vickers et al 2000), while blockade of 5-$HT_{2A/2C}$ receptors increases food intake and body weight gain (Currie et al 1996). Anorectic effects of d-fenfluramine (a 5-HT releaser and uptake inhibitor) are reduced in (obese) 5-HT_{2C} receptor deficient mice (Vickers et al 1999). Recently it was shown that 5-

HT_{2C} receptors, which are located on pro-opiomelanocortin (POMC) neurons in the arcuate nucleus, mediate the appetite-suppressant effects of d-fenfluramine (Heisler et al 2002). In the present study we showed that olanzapine treatment decreases RWA. However it was previously shown that fluoxetine (SSRI) and 5- $HT_{2A/2C}$ agonists also decrease RWA (Altemus et al 1996; Pirke et al 1993). Regarding conflicting data on serotonergic control of RWA, the possible involvement of other neurotransmitters should not be ignored. For instance, the antagonizing effects of olanzapine on the H_1 receptor might also lead to decreased activity and increased food intake (Fukagawa et al 1989; Lozeva et al 2000). In addition, possible antagonism of the DA system causing decreased motor activity should not be disregarded, although it is generally stated that olanzapine has only limited extrapyrimidal side effects. Modulation of motor activity following olanzapine treatment, as observed in the present study, might thus be related to an altered balance between 5-HT-DA-H systems.

To conclude, we showed that olanzapine treatment decreased RWA in ad libitum fed rats and rats exposed to the ABA model. Furthermore it was shown that olanzapine treatment in ABA rats partly prevented hypothermia and reduced HPA axis activation, while in ad libitum fed rats olanzapine increased food intake and body weight gain. We suggest further study on olanzapine treatment in AN patients. We already described a small open label study in which AN patients decreased hyperactivity on olanzapine treatment. In future, larger controlled studies with olanzapine treatment should be performed, carefully analyzing activity levels, body weight gain and therapeutic outcome. Taken together, the present data of olanzapine treatment in anorectic rats and preliminary data of olanzapine treatment in hyperactive AN patients, suggest that olanzapine might be an effective therapeutic agent in reducing hyperactivity and thereby improving treatment outcome of AN patients in future.

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Predictors of recovery of ovarian function during weight gain in Anorexia Nervosa

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Abstract

Objective: To investigate whether serum levels of FSH, inhibin B and anti-Müllerian hormone (AMH) can be used as predictors of recovery of ovarian function in Anorexia Nervosa after weight gain.

Design: Follow up cohort study.

Setting: Two specialized treatment centers for eating disorders, one for adolescents (twelve-seventeen years), one for adults (beyond seventeen).

Patients: Sixty-one young women (mean age 18.2 yrs) with Anorexia Nervosa.

Main outcome measures: Time to recovery of menses.

Results: Forty-two (69%) patients recovered in weight within the first year, of which only twenty four (39%) reached resumption of regular menstrual cycles. Next to weight gain itself, initial ovarian endocrine markers such as FSH, inhibin B and AMH hormone were capable of predicting chances for resumption of menses in a multivariate analysis with time to recovery as the main outcome measure.

Conclusions: Initial ovarian endocrine markers FSH, inhibin B and AMH can predict successful recovery of ovarian function in Anorexia Nervosa patients undergoing treatment to gain weight.

Introduction

Anorexia Nervosa (AN) is a serious eating disorder of unknown aetiology primarily affecting adolescent girls and young women (Lucas et al., 1991; Hoek & van Hoeken, 2003). This condition is characterized by weight loss due to voluntary restricted caloric intake and resistance toward efforts to increase body weight. Psychological symptoms consist of disturbances in attitude and perception with regard to weight and shape and an intense fear for obesity. Amenorrhea represents another diagnostic criterion of AN (APA 1904). The pathogenesis of this potentially fatal illness remains poorly understood.

A variety of starvation-induced abnormalities can be observed, such as severe malnutrition, decreased fat mass and body temperature, low resting energy expenditure and bradycardia. Endocrine abnormalities associated with undernutrition include abnormal thyroid function (Levine, 2002), hypercortisolemia (Fichter et al., 1990) and hypothalamic hypogonadism (Newman & Halmi, 1988). Ovarian quiescence is primarily due to insufficient support by gonadotropins. Recent studies have been pointing at the pivotal role of leptin - an adipocyte-secreted hormone regulated by the Ob gene - in energy homeostasis as well as other physiological processes, including reproductive functioning (Mitan, 2004). Plasma levels of leptin rapidly decrease following weight loss (Ahima et al., 1996) and both leptin and gonadotropin secretions are strongly suppressed in the acute stage of AN (Calandra et al., 2003). A threshold requirement for leptin concentrations allowing for recovery of pituitary gonadotropin secretion has been proposed (Ballauff et al., 1999).

Anti-Müllerian hormone (AMH) - also referred to as Müllerian inhibiting substance - is a dimeric glycoprotein and a member of the transforming growth factor-β family. In the human, AMH is produced by primary, preantral and early antral follicles only (Weenen et al., 2004). AMH serum levels are cycle-independent and are associated with the number of growing follicles in the ovaries (De Vet et al., 2002; Fanchin et al., 2003). Inhibins are heterodimeric glycoprotein hormones, also members of the TGF-β family. Inhibin B is produced by small antral follicles and maximum concentrations are reached during the early follicular phase of the menstrual cycle (Groome et al, 1996).

In normogonadotropic anovulatory infertility (especially in women with polycystic ovary syndrome) serum AMH levels are elevated (Jonard et al., 2005; Laven et al., 2004) while follicle-stimulating hormone (FSH) and inhibin B levels remain within normal limits (Laven et al., 2001). In patients suffering from functional hypothalamic amenorrhea, FSH levels are decreased or within low normal limits along with significantly elevated AMH and reduced inhibin B levels, suggesting incomplete FSH action at the level of the ovary (Jonard et al., 2005; Alvero et al., 1998; Couzinet et al., 1999). The hypothalamic amenorrhea associated with AN seems to be due to a prepubertal secretion pattern of pulsatile luteinizing hormone (LH) and a dysfunctional hypothalamic response to estrogen feedback (Mitan, 2004; van Binsbergen et al., 1990). Controversy remains whether normalization of leptin is crucial for the activation of the hypothalamic-pituitary-ovarian axis (Holtkamp et al., 2003).

It is well known that while some AN-women regain normal menstrual cyclicity immediately following body weight normalization, for other patients it can take additional months to years until the target weight has been reached (Herpertz-Dahlmann et al, 1996; Golden, 1997). Factors underlying these individual differences remain to be explored. The aim of the current study is to investigate the predictive value of various ovarian endocrine markers (especially AMH and inhibin B) upon initial screening for the resumption of normal menstrual cycles following weight recovery.

Materials and methods

Subjects

Patients were entered into this study after they and/or their parents gave informed consent. The clinical research protocol for this prospectively designed, cohort follow-up study was approved by the Institutional Review Board of the University Medical Center of Utrecht (UMCU). Patients were recruited in two specialized eating disorder treatment centres (clinics), one for adolescents 12-17 years (UMCU, 31 subjects) and one for adults older than 17 years (Rintveld, 30 subjects). Applied inclusion criteria were: (I) AN diagnosed according to DSM IV (APA, 1994) on the basis of a structured interview using the Eating Disorders

Examination (Cooper et al., 1989). (II) Co-morbidity restricted to depression or anxiety disorders. (III) Secondary amenorrhea. (IV) No use of steroid contraceptives. (V) Exclusion of clinical histories of concurrent illnesses. 61 patients were included, 19 patients were of the AN purging type, 42 of the restricting type.

Study protocol

After initial psychiatric assessment patients entered a structured treatment program, aimed at restoration of body weight, eating pattern, body image, normalization of anorectic cognitions and family and social functioning. Weight gain was targeted at 0.5- 1.0 kg/wk in accordance with clinical guidelines. Weight recovery was defined as a weight within the normal range for age (> SD -1.5 corresponding with a Body Mass Index (BMI) of approximately 19 kg/m^2 for adults) and target weight as the weight at which patients resumed a regular menstrual cycle (defined as three menstrual periods with three to five week intervals). Individual, group and family therapy techniques were used to change the patients' aberrant body perception and cognition.

Body weight was measured at least once a week, psychological changes were followed weekly by self reporting using a questionnaire developed to track mood changes (Profile of Mood States (POMS)) (McNair et al., 1971). The degree of underweight was calculated using BMI (kg/m^2) and BMI computed into z-scores: describing the distance in SD from the mean BMI for that age (using a software program provided by the Netherlands Organisation for Applied Scientific Research TNO), to account for differences in age and to relate the data to Dutch population references (van Buuren & Fredriks, 2001).

Blood samples were obtained by venepuncture for hormonal analysis at the time of admission.

Hormone assays

Plasma samples were stored at -80°C prior to determination. Leptin was measured using a sensitive radioimmunoassay (RIA) (Human-Leptin-RIA sensitive, Mediagnost, Tubingen, Germany), intra-assay CV of 5%, inter-assay CV of 7.6%. Serum Inhibin B was measured by immunoenzymometric assay (Serotec, Oxford, UK) with detection limit of 3.4 ng/l,

intra-assay CV 9%, inter-assay CV 15%. Serum AMH was assessed using an ultrasensitive immunoenzymometric assay (Immunotech-Coulter, Marseilles, France) with detection limit of 0.05 µg/l. Intra-assay CV of 5% and inter-assay CV of 8%. FSH was measured using a luminescence-based immunoassay (Centaur, Bayer, Tarrytown, USA) with a detection limit of 0.1 U/l. Intra-assay CV < 3% and inter-assay of 3.5-6%. Undetectable values were arbitrarily set to zero. Estradiol was measured after diethylether extraction and Sephadex chromatography using an in house competitive RIA employing a polyclonal anti-oestradiol-antibody. [2,4,6,7-^{3}H]-Estradiol (TRK322, Amersham Nederland B.V.) was used as a tracer following chromatografic verification of its purity. The lower limit of detection was 20 pmol/l (2 ml sample) and inter-assay CV was 12 and 3% at 80 and 660 pmol/l, respectively.

Data analysis

After a follow-up of 1 year, the patients were divided into 3 groups according to their clinical stage (Table I). Data of clinical and endocrine parameters by group are presented as the mean ± SD if distributed normally, or otherwise as the median and range. Kruskal-Wallis tests were used if data were not normally distributed. In case data were normally distributed ANOVA was used. $P \leq 0.05$ was considered to be statistically significant.

To determine the associations of clinical and endocrine parameters with the rate of recovery of the menstrual cycle over time, univariate and multivariate Cox proportional hazards analyses were performed. This method of analysis estimates a linear regression model of parameters against the logarithm of the hazard (or: instantaneous risk) of the menstrual cycle recovery during follow-up. Initial values of parameters were used as potential predictors. Parameters with univariate $P < 0.30$ were candidate predictors for the multivariate model and backward elimination of parameters (with $P < 0.05$ for inclusion) was used to determine the set of most predictive parameters. Because of potential differences in average prognosis between the two clinics, the analysis was stratified by clinic. The ability of the resulting multivariate model to discriminate between fast recovering patients and slow or non-recovery was assessed by the c (or: concordance)-statistic, separately in the two clinics (Harrell et al 1996). The c-statistic (or: area under the ROC curve (AUC)) may be interpreted as the probability that the model will correctly identify the patient with the best outcome, for any given random set of

two patients. The lowest c-value is 0.5, in which case the model does no better than a random guess.

A 10-fold cross-validation procedure was applied to correct the model for over fitting and to assess the amount of optimism in the c-statistic, caused by constructing and evaluating the model on the same dataset (Harrell et al., 1996). Statistical analysis was performed using SPSS for Windows (release 11.5, SPSS Inc., Chicago, IL) and S-plus software (MathSoft, Inc., Seattle, WA, version 2000).

Results

Mean age of the patients was 18.2 ± 3.1 (SD) yrs, average BMI at admission 15.4 ± 1.3 kg/m^2 corresponding to a BMI z-score of -3.8 ± - 1.6 (Table I).

Table I: Demographics (mean + SD) of 61 young women diagnosed with anorexia nervosa participating in this follow up cohort study. Data are presented for the entire group, and separately for women presenting with no weight recovery, weight recovery only or weight + cycle recovery during an observation period of maximum 12 months.

Variables	Total	No weight recovery, Amenorrhea (NWR) (n=61)	Weight recovery, Amenorrhea (WR) (n = 19)	Weight + cycle recovery (WCR) (n = 18) (n = 24)
Age (years)	18.2 ± 3.1	17.3 ± 2.2	16.9 ± 3.2	19.8± 3.0[a] vs. NWR+ WR
Weeks in study	33.5 ± 11.4	32.8 ± 11.6	36.9 ± 14.0	31.6 ± 8.8
Bodyweight initial (z-scores)	-3.8 ± 1.6	-4.2 ± 1.9	-3.2 ± 1.5	-3.8 ± 1.2
Bodyweight initial (BMI)	15.5 ± 1.2	14.9 ± 1.3	15.7 ± 1.2	15.7 ± 1.0
Bodyweight end of study	-1.3 ± 1.1	-2.1 ± 0.8[a] vs. WCR+ WR	-0.9 ± 0.5	-0.9 ± 0.6
Amenorrhea (months)	24.4 ± 22.8	20.5 ± 13.5	30.0 ± 30.5	23.9 ± 23.6
AN Restrictive (%)	69	79	72	58
Neuroleptics (%)	57	47	55	58
SSRI's (%)	33	26	22	50

[a] $P < 0.01$

Within the time frame of one year, 42 (69%) patients presented with weight recovery (Fig. 1), of which 24 (39% of total, 57% of the weight recovered group) also exhibited recovery of menstrual cycles. In contrast, 18 patients recovered in weight but without the resumption of a menstrual cycle (Fig. 2). Nineteen (31%) patients remained at a low weight throughout the year.

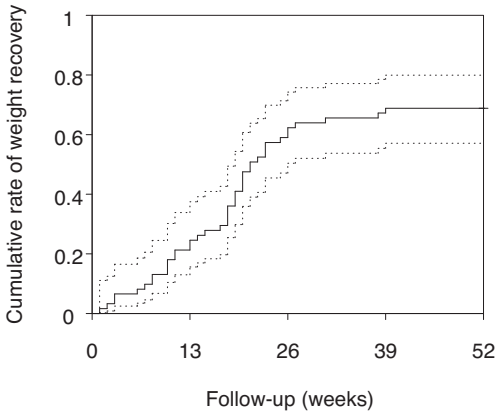

Figure 1. Cumulative chance of weight recovery (n = 61) against follow up time in Anorexia Nervosa during treatment to gain weight. The time until weight recovery was defined as the time between inclusion in the study and the time at which a BMI z-score of –1.5 or higher was reached. Dotted lines represent 95 CI.

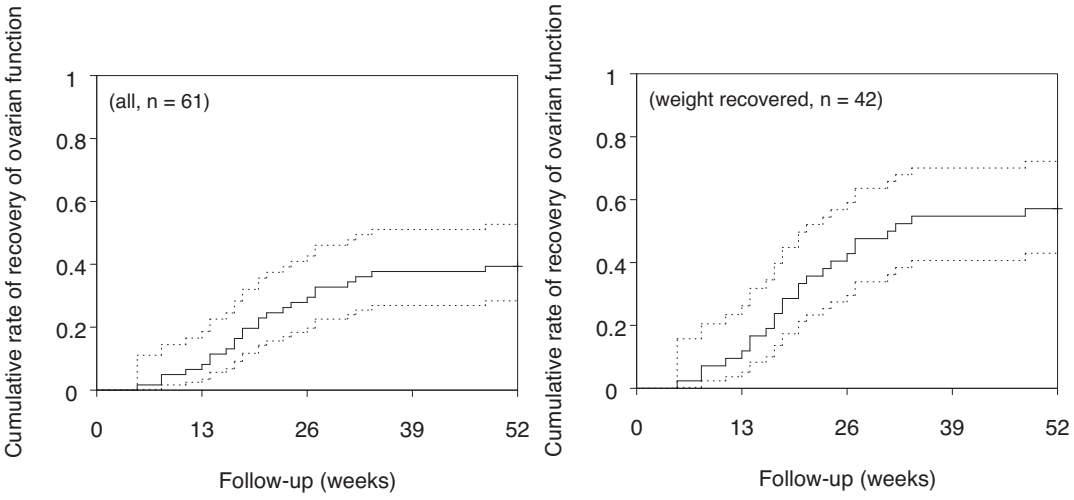

Figure 2. Cumulative chance of recovery of ovarian function against follow up time in Anorexia Nervosa during treatment to gain weight. Overall (n = 61, upper panel) and in the subgroup of patients that achieved weight recovery (n = 42, lower panel). Dotted lines represent 95 CI.

Between the outcome groups there were no significant differences in initial body weight, duration of illness as defined by duration of amenorrhea, duration of study participation, type of AN (restrictive or purging type), premorbid weight and amount of prescribed neuroleptics or SSRI's (Table I). A difference in age between the groups was observed. The group that recovered in weight and resumed a menstrual cycle was older, also corresponding with the clinic they had been treated. Chances for weight and cycle recovery differed between the two clinics, for the adolescent 16 and the adult group 63%, respectively.

Initial endocrine characteristics of the entire group and of the separate outcome groups are presented in Table II.

Table II: Initial endocrine characteristics (median and range) of 61 young women diagnosed with anorexia nervosa participating in this follow up cohort study. Data are presented for the entire group and separately for women presenting with no weight recovery, weight recovery only or weight + cycle recovery during an observation period of maximum 12 months.

Variables	Total	No weight recovery (NWR) Amenorrhea	Weight recovery (WR) Amenorrhea	Weight + cycle recovery (WCR)
	(n=61)	(n = 19)	(n = 18)	(n = 24)
FSH (IU/l)	2.8 (0.1-9.3)	2.8 (0.8-7.2)	2.0 (0.9-6.6)	3.8 (0.6-9.3)
E_2 (pmol/l)	45 (19-260)	40 (19-120)	40 (19-130)	80 (39-260)[a vs. NWR + WR]
Leptin (µg/l)	2.1 (0.5-13.3)	2.9 (0.7-8.6)	1.5 (0.5-4.6)	2.4 (0.7-13.3)
Inhibin B (ng/l)	28.0 (0.0-219.0)	28.0 (0.0-93.0)	20.5 (0.0-66.0)[a vs. WCR]	34.5 (0.0-219.0)
AMH (µg/l)	5.2 (1.1-21.7)	4.4 (1.1-14.3)	4.5 (1.2-17.2)	6.1 (1.8-21.7)

[a] $P < 0.05$

Differences in initial hormone levels between the outcome groups reached statistical significance for E_2 and inhibin B, although there was a large overlap of the concentrations in the various groups. AMH showed no undetectable samples, inhibin B 7 (11.5%): 2 in the recovered group, 5 in the group that showed no recovery. Initial E_2 levels correlated both with FSH ($r = 0.47$, $P < 0.001$) and inhibin B ($r = 0.54$, $P < 0.001$). Inhibin B correlated with FSH ($r = 0.46$, $P < 0.001$). Initial body weight correlated with all hormones measured, except AMH ($r = 0.15$) (data not shown).

After stratification by clinic, we analyzed the predictive value of the relevant clinical and endocrine parameters on the main outcome measure: Time to recovery of the menstrual cycle (Table III). Both endocrine parameters (leptin, FSH, E_2, inhibin B, AMH) and BMI z-score predicted a favourable outcome. A leptin threshold level could be detected using a 4-knots restricted cubic spline function for initial leptin in the Cox analysis (Harrell et al., 1988); A strong positive association between the leptin level and the chance of recovery was observed, up till a leptin concentration of 2.0 μg/ml, with no further increase in chance above that level (data not shown). However, in a multivariate analysis, leptin lost it's significance and initial inhibin B, AMH and FSH levels showed the strongest relationship with recovery of the menstrual cycle, with a c-statistic (corrected for optimism using cross-validation) of 0.80 (95% CI: 0.71-0.89) and 0.75 (95% CI: 0.57-0.92) for the adolescent and adult group respectively.

Table III: Association of clinical and endocrine features upon initial screening with time to recovery of ovarian function.

Parameter	Univariate P-value	Multivariate P-value	Hazard Ratio (95% CI)[a]
BMI z-score	0.014		
Age	0.8		
FSH	< 0.001	0.006	1.91 (1.20 – 3.03)
E_2	< 0.001		
Leptin	< 0.001		
Inhibin B	< 0.001	0.008	2.06 (1.21 – 3.52)
AMH	< 0.001	0.012	1.74 (1.13 - 2.69)

[a]Hazard ratio for standardised parameters, with 95% confidence interval. The hazard ratio represents the change in hazard when the parameter is increased by one standard deviation.
Cox regression stratified by clinic.

Discussion

From the literature it is known that the outcome of AN is rather poor (Zipfel et al., 2000; Russell et al., 2001; Steinhausen 2002). Approximately 20 – 30 % of patients remain chronically ill, another 30- 40% reach an intermediate level sufficient to return to a productive level of functioning but with continued struggles around weight and shape and another 40% recover with few sequelae. Resumption of the menstrual cycle represents an important step in the recovery of AN. However, the conditions that enable recovery from ovarian quiescence remain poorly understood. Weight gain is a prerequisite for re-establishing normal menstrual cycles. Even so, some patients recover in weight but resumption of normal ovarian function can take additional months to years.

Misra and colleagues (Misra et al., 2006) found that higher baseline cortisol levels predict increases in body fat, which in turn predict menses recovery in AN patients. The focus of the current prospective follow up study in young AN women undergoing intervention to gain weight was to investigate for the first time whether novel ovarian markers such as inhibin B and AMH upon initial screening would allow predicting chances for ovarian recovery. It was found that initial serum concentrations of FSH, inhibin B and AMH are important factors predicting chances for recovery of normal ovarian function. The area under the receiver operating characteristic curve of the multi-variate prediction model (i.e. the c statistics) is 0.8 and 0.75 for adolescent and adult patients, respectively. Hence, despite the modest difference in initial concentrations, the combined model is fairly accurate in predicting chances for menstrual cycle recovery in women with body weight normalisation.

Weight gain represents the first step in the struggle for recovery from AN. In the current study, 69% of patients reached a weight within the normal range (for their height and age) within one year of treatment. These figures are in line with previously published observations (Zipfel et al., 2000; Russell et al., 2001; Steinhausen 2002). Roughly half of this group regained menstrual cyclicity within the same time period. The exact extent of weight gain needed for full recovery of ovarian function is uncertain. Pelvic ultrasound scanning after short term weight restoration has shown that ovarian size is not normalized in most anorectic patients with a BMI below 17.8 kg/m^2 (Sobanski et al., 1997). Often a near 100% weight-to-height restoration is required, and persistent amenorrhea simply results from a relative subnormal weight. Individual differences in serum leptin levels resulting from weight gain may also prevent some patients to achieve full ovarian recovery (Hebebrand et al., 1997; Holtkamp et al., 2004). The current study shows that initial leptin levels do correlate with the chances for recovery of ovarian function and a threshold value could indeed be established. However, after correction for a possible association with other endocrine markers by means of multi-variate analysis, leptin disappeared as a predictor.

Psychological factors may also play a role in chances for recovery of normal ovarian function. Remaining anorectic fears and distorted perceptions represent ongoing underlying aberrant neuropeptidergic signalling that result in altered serotonin levels and persisting alterations in brain functioning Kaye et al., 1991; Barbarich et al., 2003).

Unfortunately, currently available tests concerning state and trait characteristics are incapable of differentiating AN women with a good or poor prognosis. Clinically, we need markers to enable us to predict more accurately who of our patients will have the possibility to reach a normal level of psychological functioning within a reasonable amount of time.

As expected, E_2 and FSH levels were below normal at the start of the study in all patients. Inhibin B levels were also low reflecting the prepubertal stage of these patients, and indicating a reduction in number of follicles at a more advanced stage of follicle development. AMH levels were within the normal range for age reflecting unaffected noncyclic, gonadotropin-independent growth of small preantral and early antral follicles. Only a single cross sectional study previously focused on inhibin B in AN (Popovic et al., 2004). It was observed that inhibin B levels normalised only in AN patients presenting with both weight and menstrual cycle recovery and it was suggested that inhibin B may be a useful marker for resumption of ovarian activity. Several previous studies focussed on ovarian endocrine markers in relation to ovarian physiology (De Vet et al., 2002; Crofton et al., 1997) and pathophysiology (Laven et al., 2001;Anderson et al., 1998; Bath et al., 2003), but up till now AMH has not been assessed in the context of anorexia nervosa. AMH levels were found to be slightly elevated compared to controls in women presenting with functional hypothalamic amenorrhea (Jonard et al., 2005), a condition comparable to ovarian quiescence in AN patients.

In conclusion, the current prospective cohort follow up study in AN patients undergoing a structured weight gain program demonstrates that ovarian endocrine markers such as FSH, inhibin B and AMH upon initial screening are capable of predicting chances to regain normal ovarian activity in case body weight is normalized. Hence, in case ovarian function is suppressed less profoundly at the start of an intervention in AN patients to gain weight, chances are increased that weight recovery will indeed also result in menstrual cycle recovery. In addition, the dynamics of these markers over time and their interrelationship should be assessed. This approach may eventually aid in more patient tailored treatment strategies in AN patients.

Conflict of interest: none

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Changes in mood states during recovery from anorexia nervosa

7

Abstract

Background: In Anorexia Nervosa (AN), recovery is a complex issue. Physical recovery like return of menses is often not accompanied by recovery of psychological symptoms of AN such as distorted attitudes to food and appearance, depressive feelings and anxiety. In this study we investigated psychological changes during the recovery process in AN to determine how these parameters alter as a function of recovery, in patients who underwent a structured treatment program, aimed at restoration of weight, eating pattern, body image, normalisation of anorectic cognitions and family and social functioning.

Methods: Mood states were assessed weekly using the shortened version of the Profile Of Mood States scale (POMS) in two AN patient groups (adolescents and adults) from the start and over the course of treatment, up to a year.

Results: At the end of the study 41 patients had recovered in weight of which 23 (56%) showed weight and cycle recovery, 15 patients remained at a low weight. At the start of the study the overall picture of the patient group is of extremely high scores on the subscales 'depression' and 'tension', high scores on the subscale 'anger', above average scores for 'fatigue' and below average for 'vigor'. All subscales showed significant changes over time, effect sizes differed between the outcome groups. For the subscales 'depression' and 'tension' the WR group showed a significantly larger reduction. The 'anger' subscale showed a significant reduction in all groups, and although effect sizes differed between the outcome groups this did not reach statistical significance. On the 'fatigue' subscale a significant reduction was visible overall, with similar results for the WCR and WR outcome groups, but the NWR group showed an increase in symptoms instead of a reduction. The 'vigor' subscale showed the opposite picture: a significant positive effect over time, similar in the WCR and WR group, but less for the NWR group, reaching statistical significance.

Conclusions: Over all physical recovery is accompanied by changes in mood states. Restoration of weight or even full physical recovery including resumption of a menstrual cycle however is not straightforward followed by a normalisation of mood. After nine months in treatment for AN, anxiety and depression symptoms scores remain in the clinical range, and would require treatment in itself.

Introduction

Recovery of anorexia nervosa is often based on physical criteria, like return of menses and normalized body weight but individuals considered to be physically recovered often continue to show distorted attitudes to food, eating, and weight (Clinton & McKinlay, 1986). Within the eating disorder literature clinicians have long recognized the importance of psychological dimensions of recovery from eating disorders (Bruch, 1974, 1982) but psychological markers for physical recovery have not yet been found. Most follow up studies are based on the Morgan-Russell Global Assessment Score (MRGAS) (Morgan & Hayward, 1988), which in turn is based on the Morgan-Russell Outcome Assessment Schedule (Morgan & Russell, 1975). Although this schedule originally contains five subscales, including physical status, menstruation, mental status, sexual functioning and socio-economic status, many studies only use it to describe body weight, eating habits and menstrual status (Eckert et al, 1995).

Psychological aspects of recovery, such as a reduction in fears about becoming fat, preoccupation with food, and appearance and disturbances in body image, have historically received much less attention in outcome evaluations (van der Ham et al, 1994). Researchers' definitions of recovery often refer to the absence of any disturbance in perception of body image in addition to the restitution of body weight and menses, (Eckert et al, 1995). In addition a number of authors include the patient's "social" environment (Noordenbos & Seubring, 2006; Norring & Sohlberg, 1993; Steinhausen & Glanville, 1983a, 1983b).

Other aspects of recovery are duration of improvement and relapse. Herzog et al. (1997) state that a meaningful construct of recovery needs to consider both severity of symptoms and also time to improvement. Many studies report that eating disorder patients are at substantial risk of relapse, with as many as 50% of patients relapsing within the first 3 years after treatment (Norring & Sohlberg, 1993), the endurance of a "symptom-free" state does appear an important component of a recovery definition. However, several of the studies that have incorporated "duration of wellness" into the assessment process have defined recovery as maintaining a symptom-free state for only 8 consecutive weeks (Strober et al, 1997; as quoted in Pike, 1998). This definition may be useful for research purposes, enabling between-study comparisons to be made but whether these patients are "recovered" from their eating

disorders after only 8 weeks free of eating disorder symptoms seems unclear. Indeed, many authors argue that meaningful evaluations of "recovery" from eating disorders can be made only on long-term follow-up (Kreipe et al., 1989; Pike, 1998). This discrepancy highlights the need for clarification about use of the term recovery in outcome research, and also indicates that more studies that assess change over a series of short follow-up intervals could make a valuable contribution to this area (Herzog et al., 1988).

Co morbidity should also be considered in a definition of recovery. In his review on co morbidity at follow-up Steinhausen describes 24.1 % mood disorders (2 - 67%), 25.5% anxiety disorders (4 - 61%), 12.0% OCD (0 - 23%) and 17.4 – 31.4% personality disorders. Herpertz-Dahlmann and colleagues (2001) found a significant correlation between psychiatric co-morbidity and recovery from AN after 10 years.

Physical recovery seems to be a necessary prerequisite but not the only predictor of psychological recovery (Fennig et al., 2002; Steinhausen, 2002; Strober et al., 1997). Stable physical recovery is reached after on average 4.7 years but psychosocial recovery was reached only after 6.6 years (Strober et al, 1997; Fennig et al, 2002; Eckert et al, 1995).

It seems, therefore, that a clinically relevant definition of recovery from an eating disorder needs to encompass physical, psychological, and social dimensions of change.

Aim of study

To identify psychological changes during the recovery process in AN and to determine how these parameters alter as a function of recovery we decided to study changes in mood states, in patients who underwent a structured treatment program, aimed at restoration of weight, eating pattern, body image, normalisation of anorectic cognitions and family and social functioning.

Methods and materials

Subjects

Patients were entered into this study after they and/or their parents gave informed consent. The clinical research protocol for this prospectively designed, cohort follow-up study was approved by the Ethics Review Committee of the University Medical Center of Utrecht (UMCU). Patients were recruited in two specialized eating disorder treatment centres (clinics), one for adolescents 12-17 years (UMCU, 26 subjects) and one for adults older than 17 years (Rintveld, 30 subjects). Applied inclusion criteria were: (I) AN diagnosed according to DSM IV on the basis of a structured interview using the Eating Disorders Examination (Cooper et al, 1989). (II) Co-morbidity restricted to depression or anxiety disorders. (III) Secondary amenorrhea. (IV) No use of steroid contraceptives. (V) Exclusion of clinical histories of concurrent illnesses.

Study protocol

After initial psychiatric assessment patients entered a structured treatment program, aimed at restoration of weight, eating pattern, body image, normalisation of anorectic cognitions and family and social functioning. Weight gain was targeted at 0.5- 1.0 kg/wk in accordance with clinical guidelines. Weight recovery was defined as a weight within the normal range for age (> SD -1.5 corresponding with a Body Mass Index (BMI) of approximately 19 kg/m^2 for adults) and target weight as the weight at which patients resumed a regular menstrual cycle, defined as three menstrual periods with three to five week intervals. Individual, group and family therapy techniques were used to change the patients' aberrant body perception and cognition. Patients completed the study by reaching a regular menstrual cycle as described above, or after a year of participation.

Measures

Patients completed the shortened version of the *Profile Of Mood States scale (POMS)* weekly.

POMS

The Profile Of Mood States scale is a self-rating scale, originally by McNair, Lorr, & Droppleman, (1971) with 65 affect adjectives to be rated on a 5 point scale from *not at all* (0) to *extremely* (4). Ratings are made in reference to how the respondents have been feeling during the past week. A Total Mood Disturbance can thus be obtained, and McNair et al (1992) assert this score makes clinical sense and can be presumed to be highly reliable because of the intercorrelations among the primary six POMS factors. Several adaptations of the POMS exist, a specific version for adolescents (Terry et al, 1999, 2003); a short version containing 37 items (Shacham 1983) and an abbreviated version with 11 items lacking the original six subscales (Cella et al, 1987). The short version was validated by Baker et al (2002) and Curran et al (1995) and found to have reliable Cronbach's alphas (0.76 to 0.95) The POMS is useful to study transient mood states i.e. accompanying hormonal changes like the premenstrual syndrome (Mortola et al, 1990) or as measures of distress following psychosocial interventions and finally as an indicator of quality of life in a variety of patient groups: epilepsy (Salinsky et al, 1996), arthritis (Ward, 1994) or following HIV-infection (Schag et al, 1992) or cancer therapy (Shacham 1983; Baker et al, 2002). Studies in the field of anorexia nervosa are scarce (Nakao et al, 1998; Miyasaka et al, 2003) and have not been done to assess changes during treatment. The POMS can be repeated frequently, it has been used with intervals as brief as 3 – 5 days (Little & Penman, 1989). The Dutch version (van der Ark, 1995) has 32 items, that cluster into 5 mood states: 'depression-dejection' (eight items), 'anger-hostility' (seven items), 'fatigue-inertia' (six items), 'vigor-activity' (five items) and 'tension-anxiety' (six items), it does not contain a separate confusion-bewilderment subscale. Patients rate themselves on a 5-point Likert scale in the original version, in this study however a visual analogue scale (100 mm line tests) was used instead to maximize individual objectivity towards the weekly scoring of the POMS. Several studies already assessed the feasibility and validity of this form of the POMS and found good convergent validity (Little & Penman 1989; Temple et al, 2004).

Data analysis

After a follow-up of 1 year, the patients were divided into 3 groups according to their clinical stage (Table I). Data of parameters by group are presented as the mean ± standard error if

distributed normally or otherwise as the median and range. Kruskal-Wallis tests were used if data were not normally distributed. In case data were normally distributed ANOVA was used. $P \leq 0.05$ was considered to be statistically significant. To assess changes over time while taking account of dependence between observations on the same person a Linear Mixed Effects Model was used. This consisted of an individual straight-line regression analysis, characterized on group level by the mean and SD's of the parameters intercept and slope.

Statistical analysis was performed using SPSS for Windows (release 11.5, SPSS Inc., Chicago, IL) and S-plus software (Professional Edition 6.2), (Insightful Corp., Washington DC, 2003).

Results

Demographics

Mean age of the whole patient group was 18.4 ± 3.2 (SD) years. After a follow-up of maximum one year, the patients were divided into 3 groups according to their clinical stage: no weight recovery (NWR, 15 patients), weight recovery but with ongoing amenorrhea (WR, 18 patients), and weight and cycle recovery (WCR, 23 patients). There were no significant differences among the three outcome groups in initial and premorbid body weight, duration of illness (defined by duration of amenorrhea), duration of study participation and type of AN (restrictive or purging type) (Table I). A difference in age was observed. The group that recovered in weight and resumed a menstrual cycle was significantly older than the non-recovered group, a result that corresponds with the clinic where they were treated. Rates of recovering weight and menstrual cycle differed in the two clinics: 16% of the patients in the adolescent group recovered versus 63% of the patients in the adult group.

Study participation had to be at least 3 months and ended at resumption of a regular menstrual cycle with a maximum participation length of 12 months. Menstrual status at 12 months was ascertained in 100% of the cases. Based on the number of participants as a result of premature drop outs and patients who finished the study in the WCR group only data from the first nine months were included.

Table I. Demographics (mean + SD) of 56 young women diagnosed with AN. Data are presented for the entire group, and separately for women presenting with no weight recovery and amenorrhea (NWR), with weight recovery and amenorrhea (WR) and with weight + cycle recovery (WCR) over a period of 9 months.

Variables	Total group (n=56)	NWR (n = 15)	WR (n = 18)	WCR (n = 23)
Age (years)	18.4 ± 3.2	17.7 ± 2.3	16.9 ± 3.2	20.0± 3.0* vs. NWR+ WR
Weeks in study	33.3 ± 11.5	31.9 ± 11.4	36.9 ± 14.0	31.4 ± 9.0
Bodyweight, premorb z scores	-.28 ± 0.96	-.28 ± 0.78	-.14 ± 0.97	-.40 ± 1.06
Bodyweight, initial z scores	-3.8 ± 1.6	-4.6 ± 2.1	-3.2 ± 1.5	-3.8 ± 1.4
Bodyweight initial (BMI)	15.4 ± 1.3	14.6 ± 1.2	15.5 ± 1.2	15.8 ± 1.2
Amenorrhea (months)	24.5 ± 23.5	18.1 ± 14.9	30.0 ± 30.5	25.6 ± 23.8
AN Restrictive type (%)	68	80	72	57

*p<.01

POMS

At the start of the study, subscale scores were related to the known age and sex matched norm scores. The overall picture of the patient group is of extremely high scores on the subscales 'depression' and 'tension', high scores on the subscale 'anger', above average scores for 'fatigue' and below average for 'vigor'.

Analysis of the initial measurement shows that the WCR group scored significantly higher than the other two groups (p<.05) on the subscale 'fatigue' and the WR group scored significantly lower than the WCR group (p<.05) on the subscale 'tension' (table II).

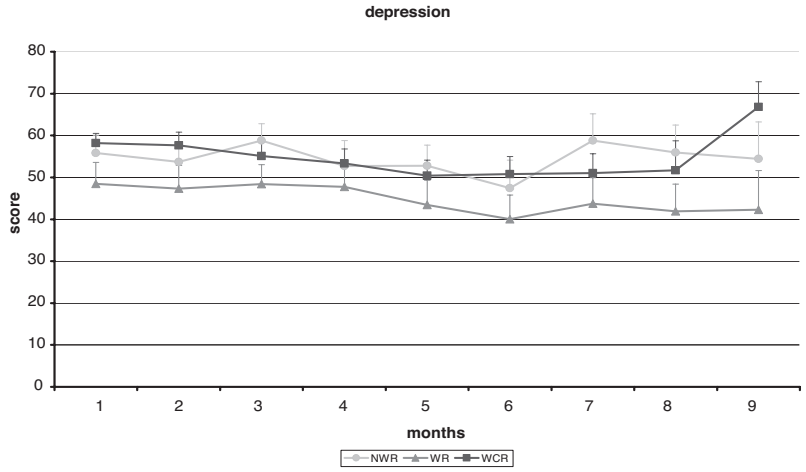
End scores of the subscales for the total group and the separate outcome groups are also given in table II. Although scores on the subscales 'anger', 'vigor' and 'fatigue' improved, end scores on the subscales 'depression' and 'tension' are still in the clinical range.

Table II. Initial and end norm scores on the Profile of Mood States (mean + SD) of 56 young women diagnosed with AN. Data are presented for the entire group, and separately for women presenting with no weight recovery and amenorrhea (NWR), with weight recovery and amenorrhea (WR) and with weight + cycle recovery (WCR); group values set against values of normal controls.

	Initial	SD	End	SD	Norm score initial[b]	Norm score end[b]
Depression: total	54.6	16.2	53,8	21,8	extr. high	extr. high
NWR	56.2	15.3	54,4	15,1	extr. high	extr high
WR	48.5	21.2	42,3	21,8	very high	high
WCR	58.2	11.1	54,6	16,2	extr. high	extr. high
Anger: total	35,7	15	28,9	13,7	high	average
NWR	37,9	14,7	35,8	16	high	high
WR	34,4	14,4	25,7	9,2	high	average
WCR	35,2	14,5	27	14,5	high	average
Fatigue: total	28,6	11,3	27,2	7,3	> average	> average
NWR	20	15,4	22,4	14,2	average	average
WR	24,8	12,7	21	15	average	average
WCR	36,7*vs NWR, WR	15,1	36,8	15	> average	> average
Vigor: total	23,5	5,2	25,3	8,3	< average	average
NWR	26,6	8,6	27,7	13,1	average	average
WR	24	8,6	20,8	6,6	< average	low
WCR	21,2	8	24,7	10,1	low	average
Tension: total	41.7	12.8	40,7	13,8	extr. high	very high
NWR	44.4	13.5	42,1	4,6	extr. high	extr. high
WR	34.9*vs WCR	15.3	32,4	16,4	high	high
WCR	45.0	7.9	49,6	7,2	extr. high	extr. high

[b]Normgroups: extr. high, very high, high, > average, average, < average, low, very low, extr. low, *p<.05

Figure 1 shows the monthly mean group values of the subscales for the different outcome groups, using visual analogue scores; the y-axis scale depicts the maximum scores for the subscale.


depression
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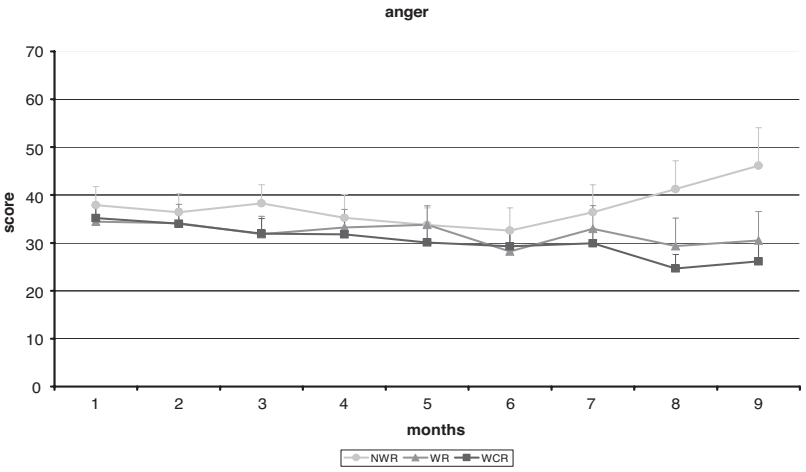
WCR

A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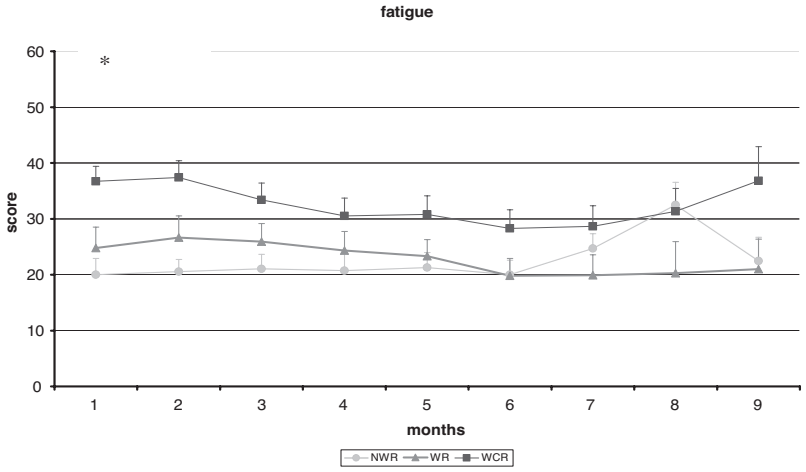
fatigue
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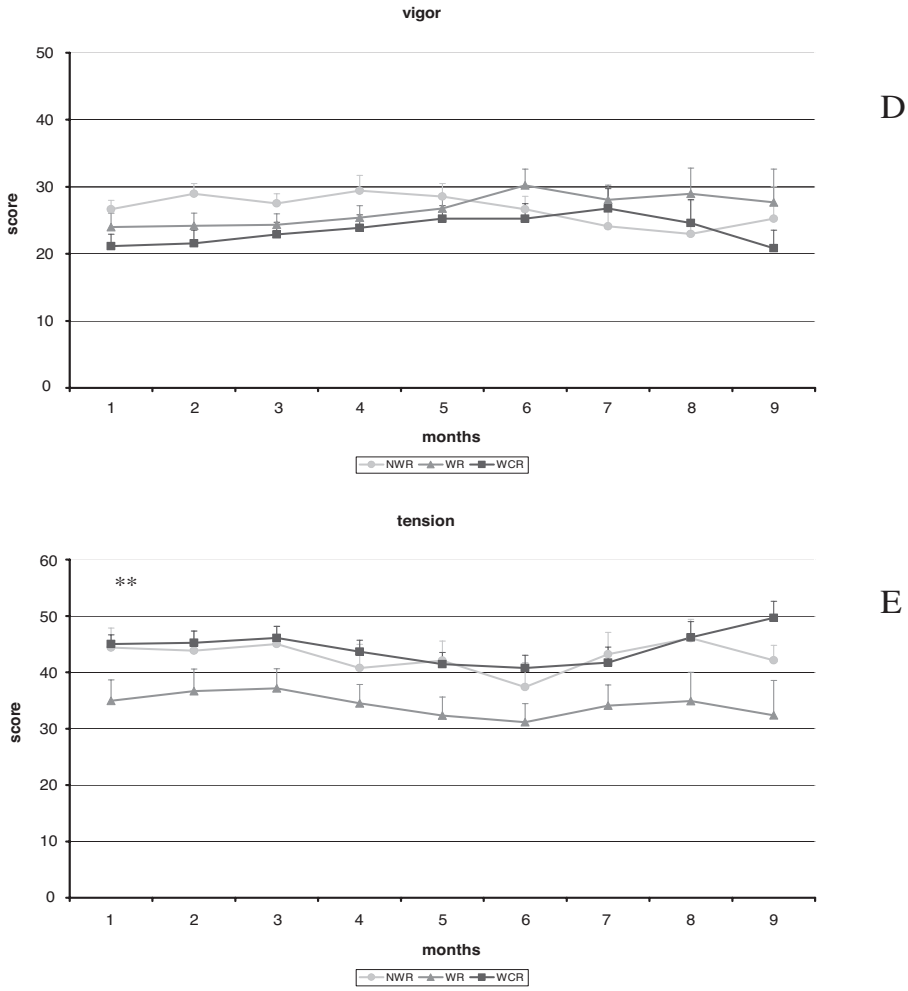

Figure. 1 A – E. POMS subscales: depression (panel A), anger (panel B), fatigue (panel C), vigor (panel D) and tension (panel E) (mean ± SE) of 56 young women diagnosed with AN participating in this follow up cohort study. Data are presented for NWR, WR and WCR over a period of 9 months.
*p<.05 WCR vs. NWR & WR, **p<.05 WCR vs WR, for differences in initial mean values.

The repeated observations were then analyzed, using an individual straight-line regression analysis, characterized on group level by the mean and SD's of the parameters intercept and slope, for changes during the months of treatment towards recovery (table III). All subscales showed significant changes over time, decreased levels on the subscales 'anger', 'depression'

'tension' and 'fatigue' and increased levels on the subscale 'vigor'. On group level, effect sizes differed between the outcome groups. For the subscale 'depression' there were no significant differences in reduction of symptoms between the NWR and the WCR group, but the WR group showed a significantly larger reduction. The 'anger' subscale showed a significant reduction in all groups, and although effect sizes differed between the outcome groups this did not reach statistical significance. On the 'fatigue' subscale again a significant reduction was visible overall, with similar results for the WCR and WR outcome groups, but the NWR group showed an increase in symptoms instead of a reduction. The 'vigor' subscale showed the opposite picture: a significant positive effect over time, similar in the WCR and WR group, but less for the NWR group, reaching statistical significance. The subscale 'tension' showed a small decrease overall, with a relative excess of effect reaching statistical significance in the WR group only.

Table III. Changes over time in subscales of the Profile Of Mood States (mean + SD, raw data) of 56 young women diagnosed with AN, using a straight-line regression analysis in a linear mixed effects model. Effect size data are presented for the entire group and separately comparing relative excess of change between groups for women presenting with no weight recovery and amenorrhea (NWR), with weight recovery and amenorrhea (WR) and with weight + cycle recovery (WCR) over a period of 9 months.

Variables	Change over time, effect size (SD) & p-value					
	Average total group		WCR-NWR		WCR-WR	
Depression	-.29(0.04)	<0.001	0.01(0.12)	0.94	0.24(0.10)	0.018
Anger	-.19(0.04)	<0.001	-.01(0.11)	0.93	0.15(0.09)	0.12
Fatigue	-.15(0.04)	<.0001	-.45(0.10)	<.0001	0.0005(0.09)	0.99
Vigor	0.11(0.03)	<0.001	0.23(0.07)	0.001	-0.05(0.06)	0.46
Tension	-.13(0.03)	<0.001	0.08(0.07)	0.28	0.14(0.06)	0.03

Discussion

This study describes changes in mood states such as 'depression-dejection', 'anger-hostility', 'fatigue-inertia', 'vigor-activity' and 'tension-anxiety' during the first nine months of recovery from AN.

During these months, approximately 30% of the patients participating in the study gained little or no weight and remained at a weight level in the anorectic range (BMI <17.5 for adults). The other patients were able to gain sufficient weight to reach the normal weight range, defined as a weight higher than 1.5 SDS below average BMI for age, which corresponds with a BMI of 19 kg/m^2 for adults. Of those patients who showed restoration of weight, 51% resumed a regular menstrual cycle, thereby fulfilling criteria for complete physical recovery.
At the end of the first nine months all patients were still receiving some form of treatment for their remaining eating pathology such as distorted attitudes to food, eating, body perception and weight.
Although the POMS has been widely used in studies on transient mood states accompanying hormonal changes, no previous longitudinal research using the POMS in AN could be found. With regards to the reliability of the self-reported mood states, several studies (Cooper et al, 1989; Fairburn & Beglin, 1990) have shown that self-report measures for eliciting and defining eating disorder symptoms are prone to bias and inaccuracies and inferior to clinical interviews. Rohde et al (1997) show that agreement varies according to the psychological problem that is being assessed. Their study agreement was excellent for self report of anxiety disorders and very good for depressive disorders; therefore the data are considered reliable.

The results make it clear that over all physical recovery is accompanied by changes in mood states. Restoration of weight or even full physical recovery including resumption of a menstrual cycle however is not straightforward followed by a normalisation of mood. After nine months in treatment, anxiety and depression symptoms scores are in the clinical range, and would require treatment in itself. These results are in line with previous outcome studies (Steinhausen 2002, Clausen 2004, Windauer 1993).

The physical differences found between the WCR and the WR group in hormonal recovery (chapter 2) are, except for the subscale 'vigor', also not paralleled by the POMS

findings. On the whole the WR group even shows slightly better scores on the 'depression', 'fatigue' and 'tension' subscales. Changes from the initial to the end levels between the two weight recovered groups are not significantly different, except for the subscales 'depression' and 'tension' where the rate of change for the WCR group scores significantly greater than in the WR group. With weight normalisation one would expect an improvement in energy level; interestingly, the subscale 'fatigue' does show improvement for the two weight recovered groups but a worsening in the underweight group. After nine months of treatment, weight restoration can bring patients normalisation of scores in the subscales 'anger', 'fatigue' and for the WCR group also 'vigor', but leaves them still anxious and depressed.

Jarman & Walsh (1999) state that in addition to lack of consensus on evaluations of outcome, clients perspectives on recovery and their influence on type of treatment are often missing. It seems that in the effort to obtain quantifiable measures of outcome the phenomenology of the client's experience of change is lost (Beresin et al, 1989). The few studies done on quality of life in eating disorder patients show poorer quality of life in particular in psychosocial domains, emotional reactions, social isolation and sleep (Keilen et al, 1994; Padierna et al, 2002; de la Rie et al, 2005).

The POMS seems able to track mood states in a reliable way, thereby opening the possibility to address the individual problems of patients more specifically.

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Outcome of Anorexia Nervosa: results of a 5 year follow-up study

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Abstract

We describe the findings of a 5-year follow-up study of severe anorexia nervosa (AN) patients referred to two specialized treatment centers in the Netherlands. Our aim was to evaluate their current state of illness and to identify potential predictors of recovery. We contacted patients who participated in a cohort study during the first year after admission Body weight and return of menses, as well as psychological features such as body attitude, psychopathology and mood states were investigated at 5 year follow-up. All 61original patients were contacted: 59% of the patients were weight and cycle recovered, 9.8% was only weight recovered, and 31.1% was not recovered. None of the original participants had died. Of the 61 original patients, 54 cooperated in a further examination of their clinical and psychological states. 16 respondents still met AN criteria, 12 had an eating disorder not otherwhise specified and none of the respondents suffered from bulimia nervosa. Despite the high percentage of weight recovery, many respondents manifested ppsychopathology and a severely disturbed body attitude. Respondents also frequently reported suffering from depression, anxiety, obsessive-compulsive and personality disorders, and feeling more tired and tense than years before. We found no predictors of treatment outcome other than relapse, but discovered that patients whose outcome changed from weight and cycle recovered after 1 year into not recovered after 5 years had been rated as more hyperactive at the 1 year time point. In conclusion this study shows that five years after admission, many AN patients recovered in body weight and menses. The majority of the participants however still suffer from severe psychopathology. The 5-year outcome could not be predicted by treatment outcome after 1 year, co-morbidity, or duration of illness. To predict treatment outcome and promote evidence-based treatment, more long-term follow-up studies of outcome in AN patients, with particular focus on the somatic as well as psychological parameters of the disease, are needed.

Introduction

Recovery from AN in general takes a long time; it has been reported that stable physical recovery is reached after on average 4.7 years and psychosocial recovery after 6.6 years (Strober et al., 1997; Fennig et al., 2002; Eckert et al., 1995). Final outcome figures of AN leave room for improvement. According to Steinhausen (Steinhausen 2002), 20.8% (0-79%) of AN patients remain chronically ill and 5.3% (0-22%) die as a consequence of starvation or suicide (Birmingham et al., 2005; Steinhausen 2002). Chances for recovery range from 0-92%, averaging at 46.5%. Recovery data in younger patient groups are more optimistic, reaching to 60% chance for complete recovery (Steinhausen 1997; Steinhausen 2002).

Full recovery of AN is in general defined as recovery of normal body weight for age and return of regular menstruation for at least 6 months (Morgan & Russell 1975). though normalization of anorexic cognitions and eating patterns are also important for returning to normal life (Pike 1998). Despite weight recovery, anorexic cognitions and abnormal eating patterns are still frequently observed in somatically recovered patients. Besides cognitions and eating patterns, a number of authors emphasize the importance of the patient's "social" environment in relation to recovery (Noordenbos & Seubring 2006; Norring & Sohlberg 1993; Steinhausen & Glanville 1983a; Steinhausen & Glanville 1983b). Furthermore a significant comorbidity of anxiety and affective disorders with AN is frequently noted (Halmi et al., 1991; Herpertz-Dahlmann et al., 2001; Kaye et al., 2004; Keel et al., 2005).

A meaningful construct of recovery needs to consider not only the severity of the symptoms but also the duration of improvement. Many studies indeed report that AN patients are at substantial risk of relapse. On average 35—50% of patients experience one or more relapses, depending on the follow-up time and definition of relapse (Keel et al., 2005; Norring & Sohlberg 1993; Pike 1998; Strober et al., 1997). Besides relapses into AN, a crossover to full blown bulimia nervosa (BN) is also frequently observed. Tozzi et al. described that 36% of their restrictive AN sample (ANr) developed BN, the majority within the first 5 years of the illness (Tozzi et al., 2005).

The duration of a "symptom-free" state appears to be an important component of a definition of recovery. However, several of the studies that incorporate "duration of symptom-

free state" into the assessment process define recovery as maintaining a symptom-free state for only eight consecutive weeks (Strober et al., 1997). Although this definition might serve inter-study comparisons, it seems inappropriate to label patients as "recovered" from AN after such a short symptom-free period. Indeed, many argue that meaningful evaluations of "recovery" from eating disorders can be made only after long-term (several years) follow-up (Kreipe et al., 1989; Pike 1998). Therefore, it is necessary to clarify the use of the term recovery in outcome research and initiate more studies assessing change in outcome over a series of short follow-up intervals (Herzog et al., 1988).

There are specific features of the illness that seem to influence outcome. Evidence exists that an early onset and early treatment of AN lead to a better prognosis of treatment outcome, as do a lack of symptomal binging or hyperactivity (Steinhausen 2002; Strober et al., 1997; Wentz et al., 2001).

The present study was performed to investigate the course and outcome of AN in 61 former adolescent patients approximately 5 years following admission to our tertiary specilized treatment centres. We examined current body weight and menses as well as current psychopathology and investigated the presence of predictors of outcome.

Materials and methods

Participants

The study sample consists of 61 female AN patients who participated in a cohort study as described elsewhere (van Elburg et al., 2007). This original study was conducted between February 2000 and January 2003 and focused on psychoneuroendocrinological changes during treatment of AN in two specialized treatment centers, one for adolescents 12-17 years (UMCU), 50.8% (n=31), and one for patients older than 17 years (Rintveld), 49.2% (n=30). Approximately five years after admission (T5) all participants were traced back and asked to participate in a follow-up study to investigate their current state of illness and psychological functioning.

At admission (=T0) all 61 participants fulfilled DSM-IV criteria of AN. Of the total sample, 68.9% (n=42) was diagnosed with restricting type AN, whereas the remaining 31.1% (n=19) was diagnosed with AN of the binge/purging type. Average age of admission was 18.2 ± 3.1 years, corresponding BMI was on average 15.4 ± 1.3 kg/m^2 (range 12.8-17.4), and average illness duration (in terms of duration of amenorrhea prior to admission) was 24 ± 22 months. Every participant was a Dutch citizen; only 2 were of non-Caucasian descent. Patients with physical or psychiatric co-morbidities other than anxiety or depression as well as male patients were excluded from joining the study at T0. The longitudinal cohort study lasted until the moment that body weight and menses were recovered (T1), with a maximum duration of one year. It was approved by the Dutch Medical Ethics Committee. All participants (and their parents in the case of minors) gave their informed consent and were informed about the possibility of a future follow-up study.

All 61 participants received clinical treatment conducted by two multidisciplinary and specialized eating disorder teams. Both teams took an integrated approach aimed at recovery of a normal body weight, eating pattern, and body attitude, as well as normalizing family relations and further development of social skills at an inpatient and/or outpatient level.

Follow-up measurement

Approximately 5 years after admission, all 61 participants of the longitudinal cohort study were informed about a follow-up study by an announcement letter mailed to their last known address. This letter gave an e-mail address which participants could use if they did not want to be further contacted. Thereafter participants were contacted by phone for further explanation of the study and were asked to participate. A set of (self-report) questionnaires was mailed to participants who agreed to take part to investigate the current state and course of their AN. If forms were not returned within 3 weeks, participants were contacted again. Participants who did not wish to take part in the follow-up were asked for their reasons as well as their current state of illness.

The test battery consisted of several items to assess Morgan Russell criteria for body weight recovery and regular menstruation (Morgan & Russell 1975). Good outcome was defined as cyclic menstruation and a minimal BMI of 18.5 (T5) or BMI computed into z-scores (T1) (van Buuren & Fredriks 2001) (WCR: weight and cycle recovered). Intermediate outcome was defined as a minimal BMI of 18.5 without (regular) menstruation (WR: weight recovered). Poor outcome was defined as BMI below 18.5 and absence of menstruation (NR: no recovery).

When respondents gave no specific information about their body weight, an estimation of BMI was made by the principal researcher (AvE), based either available information from the records or on visual examination of the respondent at the reunion of study participants a few months later. Body weight data from these respondents were used to calculate BMI categories only. All respondents were asked about their use of oral contraception. If respondents used oral contraception and reported menses, their BMI values were first examined to judge whether a natural menses would be possible. Secondly, their BMI and menstrual state at T1 (when contraception was prohibited) were compared with their BMI at T5. Once a respondent had regular menses at T1 and BMI at T5 was consistent with BMI at T1, a natural menses was assumed.

Respondents were asked about current treatment and the existence of other eating disorders or other psychiatric problems. Eating behavior, anorexic cognitions, body attitude (Dutch version of Body Attitude Test, BAT) (Probst 1995), happiness (subjective grades: 0= very unhappy, 10=very happy), social and occupational adjustment, as well as general psychopathology (Dutch version of Symptom Checklist-90, SCL90, (Arrindell 2005) and current mood state (Dutch version of Profile of Mood State, POMS, (van der Ark LA 1995) were investigated. The POMS data were scored on a visual analogue scale and converted to stanines to obtain high and low scores. In this paper only raw scores are presented (Little & Penman 1989). We also asked whether respondents had experienced a relapse (defined as a body weight below BMI 18.5 after achieving a significant initial response, and/or as amenorrhea) in the period up to T5. Finally we investigated whether participants' (log) leptin levels and nurse ratings of their hyperactivity at T1 (Van Elburg AA 2007) were predictors of 5 year outcome.

Statistics

The participants were divided into three categories of recovery. In this paper, data will be presented for all three categories, as well as for the two weight recovered categories combined (when there was no significant difference between WCR and WR).

The program SPSS 14.0 was used for statistical analysis. Normal distribution of all data was investigated. Descriptive analyses, ANOVA, t-tests for paired values or independent values or non-parametric Mann-Whitney U statistics were performed. For correlation analysis Pearson coefficients and chi-square statistics were evaluated. Stepwise linear regression was performed to investigate putative predictors of outcome. A p value of <0.05 was considered significant.

Results

Traceability:

We collected information on body weight and menses recovery of all 61 former patients (100% traceability) on average 4.8 ± 0.8 years after admission. A further investigation of psychological features was performed in 88.5% (n=54) of the participants ('respondents'), at T5. The remaining 11.5% (n=7) of participants ('rejectors') did not want to take part further for several reasons (they were still suffering severely from AN (n=2), were currently healthy and did not want to be remembered of AN (n=4), or for unknown reasons (n=1)). The 'rejectors' did not differ from the 'respondents' in type of AN, age, BMI at T0, nor in outcome at T1 or T5 (data not shown).

Criteria of outcome: BMI and menses

At admission to the treatment centers (T0), participants fulfilled DSM-IV criteria for AN. At the end of the cohort study (T1), on average 33 weeks later, 39.3% (n=24) of participants were recovered in body weight and menstruation (WCR), 29.5% (n=18) recovered in body weight but still showed amenorrhea or menstrual irregularity (WR), and 31.1% (n=19) had

not recovered in body weight or menstruation (NR) (Figure 1, (van Elburg et al., 2007)). At five years follow-up (T5), 59.0% (n=36) of them had a normal body weight and menstruation (WCR), 9.8% (n=6) had a normal body weight but showed lack of menstruation or menstrual irregularity (WR), and 31.1% (n=19) had a subnormal body weight or menstruation (Figure 1). Hence, outcome at T1 was not stable. Figure 1 depicts the shift of patients among the three outcome categories; it shows that 42% of patients who were labeled NR at T1 developed into WCR after treatment.. However, 26% of the patients labeled WCR at T1 have a poor outcome at T5. Interestingly, it appeared that these patients were rated as more hyperactive at T1 compared to patients with persistent WCR outcome ($t(19)=2.894$, $p<0.009$), whereas they did not differ in any other parameter.

Average BMI at T5 was 18.6 ±3.2, which was significantly higher than BMI at T0 but not BMI at T1 ($z=-4.929$, $p<0.001$, $z=-0.175$, n.s.). Average BMI of the WCR and WR group at T5 was 20.2±2.3, versus 15.5±2.1 of the NR group ($z=-5.546$, $p<0.001$). At T5 30.6% (n=15) of 54 respondents still showed an extreme underweight (BMI<17.5) (Table 1). Outcome at T5 (BMI and menses) was not related to the subtype of AN at T0, age at T0, the original treatment centre, duration of illness, or co-morbidity at T0 (data not shown). BMI at T5 was related to BMI at T1 ($r=0.290$ $p=0.04$), although the association was rather weak.

In total, 59% of the respondents had a natural menses at T5. As of its definition, all members of the WCR group showed regular menses (although some used oral contraceptives). Seven other participants reported menses and oral contraceptives use, however, the existence of natural menses in these participants was highly unlikely because of their low BMI. (BMI<17.5). In one respondent, natural menses could not be ascertained despite weight recovery.

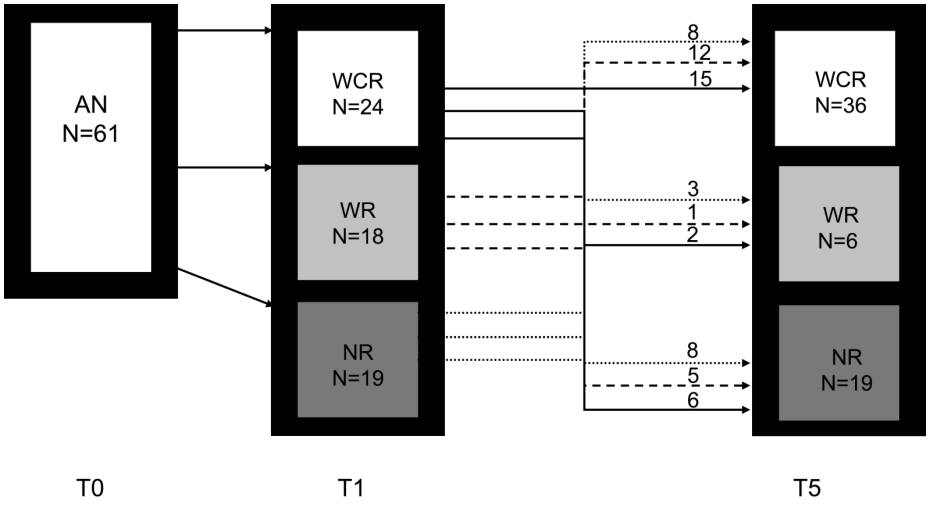

Figure 1. Outcome groups of 61 patients with AN, at start (T0) after one year (T1) and 5 years. (T5). WCR: weight and cycle recovery, WR: weight recovery, amenorrhea, NR: no weight recovery, amenorrhea.

Course of the illness: relapse, further treatment

At T5 16 (of 61) participants still fulfilled DSM-IV criteria for AN (13 ANR, 3 ANP). One of these participants crossed over from ANP into ANR. No respondents showed BN (although a history of BN was observed in one respondent) and 12 respondents showed characteristics of eating disorders not otherwhise specified (EDNOS).

The majority of the 54 respondents (72.2% (n=39) had experienced one or more relapses following remission. The experience of a relapse did not correlate with the diagnosis at T0, the outcome at T1, age, or treatment centre (data not shown), but did correlate with the outcome at T5 (χ^2=35.507, p<0.001). Respondents from the WCR and WR group experienced fewer relapses than the respondents from the NR group.

The majority of the respondents (72.2% (n=39) continued treatment for AN elsewhere in the period up to T5, ranging from individual therapy (n=17) to group therapy (n=2) and to inpatient treatment (n=8), or a combination of these (n=14). At T5, 33.3% (n=18) of the respondents were still in treatment for an eating disorder.

Respondents suffered mainly from depression (33.3%, n=18), anxiety disorders other than OCD (18.5%, n=10), OCD (11.1%, n=6) and personality disorders (9.3%, n=5). Hence,

24.1% (n=13) of the respondents started treatment for co-morbid problems after T1 and at T5 18.5% (n=10) still used treatment, ranging from low-frequency individual outpatient treatment to inpatient treatment. The frequency of current (self-reported) co-morbid problems did not differ between WCR, WR and NR groups. In addition, 37.0% (n=20) of the respondents used medication at T5, mainly selective serotonin reuptake inhibitors (SSRIs) (n=10), tricyclic antidepressants (TCAs) (n=6) and/or atypical antipsychotics (n=4).

When evaluating subjective state of the disease, 24.1% (n=13) of the respondents reported that they recovered from their disease and the remaining respondents reported that the state of the disease improved (44.4%, n=24), remained unchanged (18.5%, n=10) or worsened (13%, n=7) at T5. These subjective measurements of illness state correlated with outcome at T5 (χ^2=65.366, p<0.001), thus respondents who claimed to be recovered were mainly WCR respondents. When asked whether their current body weight was of concern, 20.4% (n=11) answered that it was still continuously of concern whereas 40.7% (n=22) of the respondents answered that it was never of concern. Furthermore, 25.9% (n=14) of the respondents answered that they never ate less than they actually should, whereas 5.6% (n=3) answered that they always ate less then they should which correlated with their outcome at T5 (χ^2=97.582, p<0.001). Interestingly, 11.9% of the W(C)R respondents reported that their body weight was continuously of concern and 62.2% (n=23) of the WCR and WR group still restricted their diet, ranging from less than half of the time (37.8%, n=14) to more than half of the time (5.4%, n=2).

Table I.

		T0	T1	T5	T5
Participants		100% (n=61)	100% (n=61)	100% (n=61[α])	88.5% (n=54[β])
Age	(SD)	18.2 (3.1)	18.9 (3.1)	23.1 (3.0)	23.0 (3.0)
Group					
	WCR	0	39.3% (n=24)	59% (n=36)	57.4% (n=31)
	WR	0	29.5% (n=18)	9.8% (n=6)	11.1% (n=6)
	NR	100% (n=61)	31.1 (n=19)	31.1% (n=19)	31.5% (n=17)
BMI		15.4 (1.3)	18.5 (1.8)		18.6 (3.2)[χ]
	<17.5	93.4% (n=58)	26.2% (n=16)	31.1% (n=19)	30.6% (n=15)
	17.5-18.5	4.9% (n=3)	14.8% (n=9)		10.2% (n=5)
	18.5-20	0% (n=0)	34.4% (n=21)	68.8% (n=42)	28.6% (n=14)
	20-25	0% (n=0)	24.6% (n=15)		36.7% (n=18)
	>25	0% (n=0)	0 % (n=0)		4.1 % (n=2)
Diagnosis					
	ANR	68.9% (n=42)	24.6% (n=15)		24.1% (n=13)
	ANP	31.1% (n=19)	6.6% (n=4)		5.6% (n=3)
	'EDNOS'	0 (n=0)	29.5% (n=18)		22.2% (n=12)
	'BN'	0 (n=0)	0% (n=0)		0% (n=0)
Amenorrhea		100% (n=61)	44.3% (n=27)	41.0% (n=25)	42.6% (n=23)

Overview of participant characteristics at admission (T0), at the end of the longitudinal cohort study (T1), and at follow-up (T5). T5 data are based on self-report. Not all participants at T5 provided BMI data. WCR: weight and cycle recovered, WR: weight recovered, NR: not recovered. [α]: total sample of participants which were contacted at T5, [β]: total sample of respondents taking part in extensive follow-up, [χ]: n=49, because exact BMI of 5 participants was not provided. ANR= anorexia nervosa restricting type; ANP= anorexia nervosa purging type; EDNOS= eating disorder Not Otherwise Specified; BN= bulimia nervosa.

Considering social adjustment, 57.4% (n=31) of the respondents reported being in a stable relationship; of these 9.3% (n=5) was married, 18.5% (n=10) was living with their partner or in separate households (29.6%, n=16), and 42.6% (n=23) reported being single. One of the respondents had given birth to a child, two more were pregnant. Furthermore, 57.4% (n=31) of the respondents had a fulltime job (or went to school), 11.1% (n=6) worked more

than 50%, and 14.8% (n=8) had no current occupation. Interestingly, social adjustment and occupational state did not differ between the W(C)R and NR group (z=-1.373, n.s., z=-1.508 n.s.).

Psychopathology, anorexic cognitions, and mood

Despite recovery of body weight (and menses) in the majority of the respondents, most respondents (including WCR) still showed significantly higher levels of (subscales of) psychopathology than norm groups of healthy (female) controls. Almost half of the respondents (48 %, n=26) score extremely high on the total severity index of the SCL90. The total severity score (z=-2.792 p=0.005) and subscales depression (DEP), somatic complaints (SOM), insufficiency in thinking and acting (IN), and suspicion and interpersonal sensitivity (SEN) differed significantly (p<0.05) between the W(C)R and NR group, as shown in Table II.

Table II SCL90 scores of 54 respondents at 5y follow-up

SCL90 at T5	Total score (n=54)	WCR (n=31)	WR (n=6)	NR (n=17)	W(C)R (n=37)	Norm group
	Mean (sd)	Mean (sd)	Mean (sd)	Mean (sd)	Mean (sd)	Mean (sd)
Total	192.8 (60.7)	179.4 (53.4)	159.9 (50.3)	229.9 (61.7)	175.7 (52.7) *	128.9 (36.4)
AGO	11.4 (5.4)	11.2 (5.3)	10.1 (3.4)	12.2 (6.2)	11.0 (5.0)	8.7 (3.4)
ANX	21.8 (8.3)	20.7 (7.5)	18.1 (8.3)	25.4 (8.7)	20.2 (7.6) *	14.6 (5.7)
DEP	40.5 (15.9)	36.4 (14.9)	32.0 (10.5)	51.1 (14.7)	35.6 (14.1) *	23.8 (8.6)
SOM	24.2 (7.8)	22.6 (5.7)	19.6 (9.1)	28.8 (8.7)	22.0 (6.5) *	18.7 (7.1)
IN	20.4 (6.5)	18.8 (5.9)	18.9 (7.0)	23.9 (6.3)	18.8 (6.0) *	14.1 (5.1)
SEN	39.7 (13.4)	36.3 (12.9)	33.7 (7.6)	48.2 (12.7)	35.8 (12.0) *	26.3 (8.8)
HOS	9.5 (3.5)	8.8 (2.3)	8.7 (4.6)	11.1 (4.3)	8.8 (2.8) *	7.6 (2.4)
SLE	6.9 (3.5)	7.2 (3.5)	3.9 (1.9)	7.8 (3.6)	6.5 (3.5)	5.2 (2.8)

AGO: agoraphobia, ANX: anxiety, DEP: depression, SOM: somatic complaints, IN: Insufficiency in thinking and acting, SEN: Suspicion and interpersonal sensitivity, HOS: hostility and SLE: sleeping problems. Norm group: healthy women who were part of a mixed norm group with average age of 43 years (range: 18-83) (Arrindell) * = sign diff from NR, p<0.05

Body attitude of the respondents was investigated using the BAT**,** and compared with a norm group of female students. Results are depicted in Table III. The majority of the respondents (70.4%, n=38) scored in the clinical range of negative body experience. The total BAT score and BAT2 score was significantly lower in the W(C)R group compared to the NR group (t(52)=2.532, p=0.001, t(52)=4.177, p<0.001). Average values of the W(C)R group were still significantly higher than values from the healthy norm group and not different from acutely ill AN patients. Moreover 65.7% of the W(C)R participants still scored in the clinical range of a disturbed body attitude.

The BAT-2 score was significantly related to BMI at T5 (r=-.335 p=0.019), was increased in participants who underwent further treatment for their eating disorder (t(52)=-2.826, p=0.007), and also related to several psychopathological subscales from SCL90 (data not shown).

Table III BAT scores of respondents at 5y follow-up

BAT at T5	Total (n=54)	WCR (n=31)	WR (n=6)	NR (n=17)	W(C)R (n=37)	Norm group Norm 1	Norm group Norm 2
	Mean (sd)	Mean (sd)	Mean (sd)	Mean (sd)	Mean (sd)	Mean (sd)	Mean (sd)
BAT Total	50.4 (21.3)	46.5 (21.2) *	42.3 (17.5)	60.8 (20.2)	45.7 (20.4)	27.6 (14.7)	42.4 (18.5)
BAT-1	16.3 (8.6)	16.1 (8.9)	12.0 (7.7)	18.4 (8.3)	15.4 (8.7)	8.4 (6.9)	8.9 (8.9)
BAT-2	15.7 (8.3)	13.0 (7.4)	12.7 (6.2)	21.8 (7.6)	12.9 (7.1)	5.8 (3.9)	4.5 (4.5)
BAT-3	11.2 (4.8)	10.4 (4.9)	10.1 (4.5)	13.1 (4.6)	10.4 (7.4)	6.7 (2.8)	11.2 (5.1)

BAT1: negative appreciation of body size, BAT2: lack of familiarity with one's own body, BAT3: general body dissatisfaction. Norm group 1: healthy women with average age of 18 (range: 12-35), Norm group 2: ANR patients who were part of a larger group of mixed eating disorders patients with average age of 24 (range: 12-35) years and BMI 17.2 (4.3) * = sign diff from NR, p<0.05

We also investigated happiness and mood states. On a grade scale from 0 to 10, respondents scored on average a `6` for happiness, with a range from 0 to 9. Respondents of the W(C)R group ranked themselves on average higher ('6.8') than NR respondents ('4.7') (z=-3.328, p<0.001).

Next, mood was investigated using the POMS, which was also used at T0 and T1 in 51 participants. Repeated measurements analysis on three POMS time samples (T0, T1, T5) of 51 participants showed that all of the subscales of the POMS except anger changed significantly over time (see Table IV). Interestingly, Table IV also shows that average values at T5 are sometimes not different or worse from the POMS data collected at T0. Paired t-tests showed that participants at T5 report significantly more vigor ($t(49)=11.431$) and less depression ($t(49)=-2.085$), but also more tension ($t(43)=3.403$) and fatigue ($t(49)=4.832$) compared to T0 ($p<0.05$). Whereas respondents at T0 did not differ in depression, anger, fatigue, vigor, or tension, W(C)R participants were less depressed ($t(52)=3.982$ $p<0.001$), less angry ($t(46)=4.182$ $p<0.001$), less tired ($t(52)= 2.020$ $p=0.049$), more tense ($t(46)=2.549$ $p<0.014$), and tended to experience more vigor compared to the NR participants at T5.

Table IV

POMS	T0 (n=51)	T1 (n=51)	T5 (n=51)#		
	Mean (sd)	Mean (sd)	Mean (sd)		
Depression	55.3 (15.4)	47.7 (19.0)	46.8 (27.6)		*
Anger	35.4 (13.5)	29.2 (13.9)	35.4 (20.6)		
Fatigue	28.3 (14.3)	24.6 (13.3)	44.8 (25.2)		*
Vigor/Activity	24.0 (7.8)	27.8 (9.5)	56.0 (19.9)		*
Tension	42.2 (12.0)	38.7 (12.6)	53.7 (21.4)		*
POMS at T5	WCR (n=31)	WR (n=6)	NR (n=17)	W(C)R (n=37)	
	Mean (sd)	Mean (sd)	Mean (sd)	Mean (sd)	
Depression	37.0 (24.2)	35.3 (21.8)	64.9 (25.5)	36.8 (23.5)	*
Anger	27.5 (17.6)	28.8 (17.8)	49.6 (18.9)	27.7 (17.4)	*
Fatigue	40.0 (24.5)	38.0 (29.9)	54.0 (22.0)	39.7 (25.0)	*
Vigor/Activity	59.7 (19.3)	57.2 (25.8)	48.8 (16.5)	59.3 (20.1)	
Tension	48.6 (21.5)	43.9 (20.1)	63.1 (18.8)	47.8 (21.1)	*

POMS data is shown of 51 participants instead of 54 participants as POMS data from 4 participants were missing at T0 and T1. * W(C)R sign different from NR, $p<0.05$

Predictors

A stepwise multiple regression analysis was used to investigate the existence of predictors of outcome of disease at T5. Except for the experience of a relapse in the period prior to

T5, we found no predictors of outcome at T5. Thus neither duration of illness, subtype of AN, BMI at T0, age at T0, comorbidity at T0, treatment center, outcome at T1, nor BMI at T1 predicted outcome of disease at T5. Furthermore, POMS scores at T0 or T1 did not predict treatment outcome at T5 and neither did (log) plasma leptin levels and nurse ratings of patients' hyperactivity at T1

Discussion

In this paper we describe the results of a 5 year prospective follow-up study on adolescent AN. Five years after admission for severe AN the majority of participants had a good outcome, concerning body weight and menses, but still a large part of them suffered severely from anorexic cognitions, anxiety and depression. The strength of this study is that we managed to contact all 61 participants that were extensively studied five years before (van Elburg et al., 2007). Information on state of illness was obtained for all participants, none of the participants died, and 88.5% of the participants agreed to take part in a more extensive follow-up to investigate psychological features.

At follow-up (T5), 59% of the participant recovered in body weight and menses (WCR), 9.8% showed a recovered body weight, but no menses (WR), and 31.1% still suffered from AN (NR). The recovery rate of our sample is comparable with the 5 yr follow-up data of Steinhausen (Steinhausen & Seidel 1993). Both studies focussed on young AN patients, which might explain the good (physical) outcome numbers. Patients in our study were on average 18.2 years old when they entered treatment at our units. It has been described previously that young AN patients (10-20 years) in general have a better outcome than older AN patients (Herpertz-Dahlmann et al., 1996; Steinhausen 1997; Steinhausen 2002; Strober et al., 1997). We found that the majority of the respondents did not meet the diagnosis of an eating disorder at T5, 16 respondents however still fulfilled the criteria for AN, and 12 respondents still showed features of an eating disorder not otherwhise specified (EDNOS) (including the WR category). A surprisingly low number (n=1) of participants developed BN after first diagnosis of AN. This is in contrast with earlier reports (Bulik et al., 1997; Keel et al., 2005; Steinhausen

& Seidel 1993; Wentz et al., 2001), but in agreement with the 5 year follow-up data by Ben-Tovim and colleagues, who found only 5% of crossover between diagnoses (Ben Tovim et al., 2001). Crossover between subtypes of AN was not frequently observed either, in contradiction with literature (Eckert et al., 1995; Eddy et al., 2002; Strober et al., 1997). However, it has to be mentioned that diagnoses of eating disorders was mainly based on self-reports.

It has been argued before that AN patients can not be considered recovered as long as psychological features of the disease have not been investigated and/or have not improved (Couturier & Lock 2006; Jarman & Walsh 1999; Pike 1998). Next to BMI and menses, we therefore investigated the respondent's body image, general psychopathology, mood state as well as social and occupational adjustment five years after admission. We observed that cognitions and psychopathology were still present in the majority of respondents, similar to earlier reports (Clausen 2004; Steinhausen 2002; Windauer et al., 1993). Even weight restored participants showed high levels of anorexic-like cognitions and psychopathology, indicating a continuation of their illness at the psychological level even though they had recovered at the physical level. For instance, body attitude was still severely disturbed in W(C)R respondents, implying (life-)long, perturbations in body appreciation and body familiarity. In fact, BAT levels of W(C)R participants were higher than from healthy controls and not different from the AN-R norm group (Probst 1995). Next to persistent elevated BAT values, the majority of the W(C)R respondents commented that they still regularly restricted their food intake. This further implies that preoccupations with eating behaviour remain present in W(C)R respondents. Probst et al. showed before that BAT values improved significantly in the majority of ED participants one year after admission to their specific eating disorder unit (Probst et al., 1999). He showed that participants who rated themselves as improved/recovered had significantly lower BAT values than participants who rated themselves as unimproved (Probst et al., 1999), which was also found in the present study. As both the Belgium and Dutch units treat severely ill patients, differences in changes in BAT levels might be explained by differences in treatment programmes, although it should be noted that timeframes of the two studies were different (1 year vs. 5 year after admission) and unfortunately our sample lacks a BAT value at admission or at T1.

Results from the SCL-90 scale showed that current psychopathological complaints include mainly anxious, depressive and somatic complaints, but also insufficiency of thinking and acting, and suspicion and interpersonal sensitivity. Even W(C)R respondents reported high levels of psychopathology. This might be explained by the fact that restoration of body weight and hormonal signalling in W(C)R respondents reduces alexithymia, and patients thus experience more positive feelings as well as negative feelings than during the period of starvation. The type of psychological complaints corresponded with (self-reported) co-morbidities. It has been shown before that a high percentage of AN patients suffers from psychiatric disorder(s) after recovery of body weight (Steinhausen 2002). Affective disorders, anxiety disorders (including OCD), personality disorders and substance abuse are among the most observed (Halmi et al., 1991; Herpertz-Dahlmann et al., 2001; Lowe et al., 2001). Our results are therefore in line with findings in the literature, although substance abuse was not reported by the respondents.

The participants' mood changed over time, but interestingly did not always improve five years after admission. Participants reported to be more tired and tense at T5 than previously, which seems paradoxical but might be explained by alexithymia in the (acute) illness state. NR participants performed worse on mood profiles than W(C)R participants. Despite the frequent presence of psychological complaints, the majority of the respondents rated themselves as happy and reintegrated in social and occupational life, and contrary to our expectations and to previous reports (Herpertz-Dahlmann et al., 2001), differences in outcome (W(C)R, NR) were not reflected in differences in social or occupational adjustment. Interestingly, we found no differences between the 31 WCR and 6 WR participants, except for the presence of menses. Thus although WR participants are not yet somatically recovered, they show similar body psychopathology, body attitude, and mood as participants who recovered both in body weight and menses. We have demonstrated before that WR participants significantly differ from WCR participants in leptin levels and body composition, which validates the use of three outcome categories. Hence, although the small number of WR participants has to be kept in mind, this study also suggests that menses per se is not needed for changes in psychological state.

As expected, a relapse experienced before T5 appeared to be correlated with the outcome at T5. We found however no other predictors of recovery in the total participant group. In contrast to Strober, hyperactivity was not identified as a predictor of outcome (Strober et al., 1997). However, W(C)R participants who changed into NR at T5 had higher activity levels at T1, as measured by trained nurses. (We have previously showed that trained nurses can reliably rate patients activity levels and that their ratings correlate with activity watch output (Van Elburg AA 2007)). Despite our earlier finding of an association between patients' age at admission and outcome at T1, we found no relationship between age at admission and outcome at T5, suggesting that the outcome at T1 was not stable (van Elburg et al., 2007).
Several possible predictors of outcome of AN have been identified before, e.g. duration of the illness, age at onset, body weight at admission, minimum weight, extent and intensity of the initial symptoms and disabilities, (duration of) hospitalization, vomiting, poor social relating or problems in the family, and personality disturbances (Ben Tovim et al., 2001; Fichter et al., 2006; Herpertz-Dahlmann et al., 2001; Lowe et al., 2001; Ratnasuriya et al., 1991; Smith et al., 1993; Steinhausen 1997; Steinhausen 2002; Strober et al., 1997; Walford & McCune 1991).

Inconsistency is, however, frequently noted, which might be due to differences between the studied patient samples as well as by small sample sizes. Unfortunately our study did not contribute in clarifying this issue as no predictors of outcome were found, however, specific phenotypical information is still very valuable for refining treatment strategies for evidence-based medicine. Therefore further studies on (phenotypical) predictors of treatment outcome using standardized protocols, objective markers, fixed follow-up times and large patient samples should be encouraged. Recently several reports have been published on the putative traits of AN. For instance, recovered AN patients still show high levels of harm avoidance, impaired set-shifting and increased striatal Dopamine receptor 2/3 binding (Frank et al., 2005; Holliday et al., 2005; Klump et al., 2004) The presence of persistent adaptations in anxiety and depression at T5 as found in our study might not only indicate continuing psychopathology, but might also indicate putative traits underlying the development of AN.

This study has several pitfalls. The interval between admission and follow-up varied between participants, with an average of 4.8 years, ranging from 3.4 to 6.1 years. However, results appeared not related to the exact time of followup (data not shown). Another pitfall

is that the data collected was mainly based on self-reports. Several studies (Cooper et al., 1989; Fairburn & Beglin 1990) have shown that self-report measures for eliciting and defining eating disorder symptoms are prone to bias and inaccuracies and therefore inferior to clinical interviews. Rohde and colleagues, however, showed that consistency varies according to the psychological problem that is being assessed (Rohde et al., 1997). For instance, agreement was excellent for self- reporting of anxiety disorders and very good for depressive disorders.

The data we gathered on eating disorder symptoms may be debatable. Although some items of eating behaviour were questioned, a standard eating disorder inventory (EDI-2) or psychiatric interview was not performed. We did, however, investigate anorexic cognitions by using the body attitude questionnaire and showed that anorexic cognitions are still present in the majority of the participants. However, the information on body weight and menses was always double checked or evaluated by an expert (AvE). The expert continues to have, even 5 years after admission, regular contact with the patients and/or their parents, and is often aware of the current illness state.

Another limitation of this study is the phenomenon of missing data. For instance, some respondents reported that they stopped weighing themselves, resulting in missing values on the parameter body weight. Another important consideration is that our results may not be extrapolatable to all AN patients. The follow-up study was performed with AN patients that were referred to our tertiary specialized eating disorder units, most often after unsuccessful treatment elsewhere, and includes only patients who also joined the longitudinal follow-up study.

Likewise, the majority of the participants also continued treatment elsewhere following treatment in our centers because the center's policy is to refer patients to regional treatment facilities once sufficient improvement has been achieved.

Finally, recovery numbers stand or fall with the criteria used. We determined recovery using two parameters, namely BMI and menstrual cycle, and classified participants as weight and cycle recovered (WCR), weight recovered (WR) or not recovered (NR). A minimum BMI of 18.5 was considered a recovered BMI and presence of regular menses was considered a recovery of menstrual cycle, based upon growth tables of healthy Dutch adolescents/adults (TNO). These criteria were also used in the first year of this longitudinal study (van Elburg et

al., 2007). Off course, different recovery numbers would have been obtained when different criteria would have been used.

Even in this relatively young sample AN has dominated many years of our study participants' lives. The impact of the illness on the patients as well as their family and finances is substantial. With this study we attempted to describe the long-term outcome of their treatment. Five years after admission, the majority of participants had a good somatic outcome, but many of them still suffered severely from anorexic cognitions, anxiety, and depression. Despite persistent psychopathology, the majority of the participants reported that they are enjoying life and reintegrated in social and occupational aspects. We unfortunately did not discover any predictors of recovery but did ascertain that one year treatment outcome was unstable. Therefore we recommend further studies with repeated measurements of AN outcome which may contribute to development of evidence-based treatments in future.

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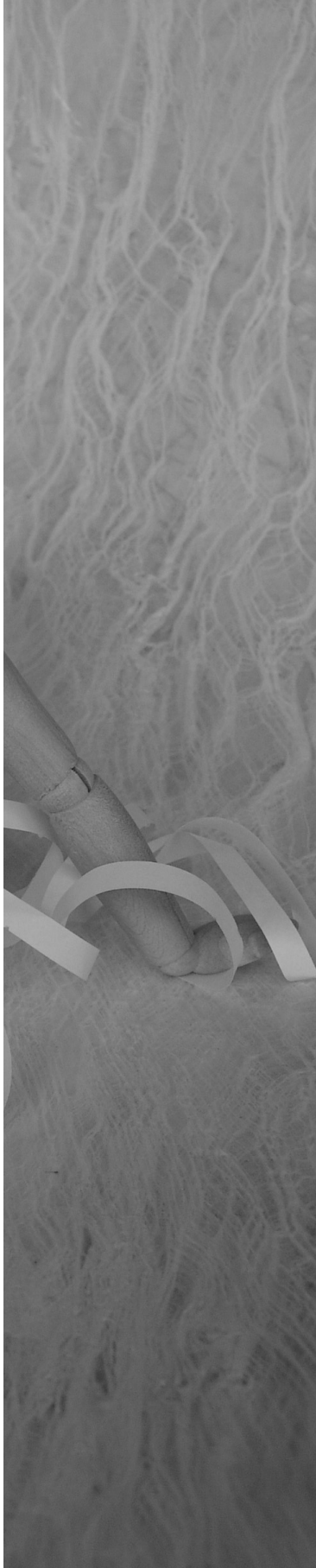
Summary, general discussion and conclusions

9



Summary

Anorexia nervosa: clinical picture

Anorexia Nervosa (AN) is a complex psychosomatic eating disorder of unknown aetiology, which primarily affects adolescent girls and young women. AN is characterized by aberrant patterns of eating behaviour and weight regulation which result in weight loss and endocrine abnormalities such as amenorrhea, disturbances in attitude and perception about weight and shape, and an intense fear of gaining weight. Patients are classified AN according to the DSM-IV when they are incapable to maintain a body weight above a minimal normal level (BMI < 17, 5 kg./m^2 for adults), with an intense fear of becoming fat; have disturbed perceptions of body shape and size and (after menarche) show amenorrhea. Two subgroups are classified, the restricting type in which weight loss is the result of dietary restriction and the binge/purge type in which periods of binging or purging and dietary restriction coexist.

The clinical syndrome of AN was described in the 19th century by Gull and Lasegue and although over the years many theories have come and gone about the origins of this disease, its core features have remained largely unchanged since the first descriptions.

AN has high morbidity and mortality rates; stable physical recovery is reached after on average 4.7 years and psychosocial recovery after 6.6 years, with frequent relapses especially in the first year after treatment. Chances for complete recovery average at less than 50% and about 20% of the patients become chronically ill. When compared to other psychiatric disorders, AN has the highest mortality rate: 10-15%.

The etiology of AN is thought to be multifactorial, a combination of a genetic vulnerability, that is triggered by environmental influences. Genetic studies have shown chromosomal regions of interest (especially on chromosomes 1,2 and 13) and have, based on association studies, also pointed at the involvement of particular genes such as several 5-HT receptors as well as AGRP and BDNF.

Hardly any evidence based treatment of AN exists and currently, state of the art treatment of AN is based upon the American and recently also the Dutch Clinical Practice Guidelines for Treatment of Eating Disorders (Trimbos, 2006) with adjustment for adolescents

in the younger age group, which mainly focuses on the involvement of the family (Lock et al., 2006; le Grange 2005).

Longitudinal study

This thesis is based upon a unique follow up cohort study, it's participants are younger than in most studies and were followed on a biweekly basis through their first year of treatment; most studies consist of observations in the acute stage and after recovery. Participants for this study were young girls and women consecutively referred to two geographically close specialized eating disorder treatment centres, one for adolescents (at the Dept. of Child and Adolescent Psychiatry of the University Medical Centre Utrecht) and one for adults (Rintveld, centre for Eating Disorders, Altrecht Mental Health Institute).

The study sample consisted of 61 female AN patients, with a mean age of 18.2 ± 3.1 (SD) years, 15.9 ± 1.2 years for the adolescent group and 20.6 ± 2.9 years for the adult group. They participated in a follow up cohort study between February 2000 and January 2003. After initial psychiatric assessment, all patients entered a structured treatment program aimed at restoration of the patient's weight, normalization of eating patterns, body image, anorectic cognitions and family and social functioning. The study focused on psychoneuroendocrinological changes during treatment; changes in body weight and composition, in hormones, activity level and mood states were carefully observed and measured. After a follow-up of maximum one year, the patients were divided into 3 groups according to their clinical status: no weight recovery and ongoing amenorrhea (NWR, nineteen patients), weight recovery but with ongoing amenorrhea (WR, eighteen patients), and weight and cycle recovery (WCR, twenty-four patients). There were no significant differences among the three outcome groups in initial and premorbid body weight, duration of illness (defined by duration of amenorrhea), duration of study participation, activity level or type of AN (restrictive or purging type) and amount of medication prescribed. A difference in age was observed. The group that recovered in weight and resumed a menstrual cycle was significantly older than the non-recovered group. This result also corresponds with the clinic where they were treated, that is, recovery rates in the adolescent clinic were lower: 16% of the patients in the adolescent group recovered versus 63% of the patients in the adult group.

To be included in the study, patient participation had to be at least 3 months and ended at resumption of a regular menstrual cycle or a maximum of 12 months, whichever came first. Menstrual status at 12 months was ascertained in 100% of the cases. Problems related to blood sampling, in combination with treatment protocol violations, turned out to be the main reason for premature dropout from the study. Based on the small number of remaining participants after these premature dropouts and of the patients in the WCR group at the end of the study, we decided to include data from the first nine months only.

Our patients behaved as could be expected from previous studies (Russell et al, 2001; Steinhausen 2002), some improved dramatically, others much less or lost motivation and left treatment prematurely. The majority of patients needed intensive and inpatient treatment, followed by day treatment and outpatient care afterwards.

Approximately five years after admission all participants were traced back and asked to participate in a follow-up study to investigate their current state of illness and psychological functioning.

Changes in body composition and hormone levels during recovery from Anorexia Nervosa

In AN food restriction leads to starvation which in turn causes changes in both central and peripheral hormonal feedback systems and associated organs. AN patients show physiological responses but also several unusual reactions in starvation conditions. In this study we investigated the changes in body composition and hormones implicated in eating behavior, menstrual cycle, and stress responsiveness in the acute stage of AN, and followed alterations in these systems over a 9 month treatment period in the patients participating in the follow up cohort study. Body weight and composition were measured at least once a week using a TANITA® body composition analyzer. Leptin, FSH, LH, estradiol, progesterone, testosterone, ACTH, insulin-like growth factor-1 (IGF-1) and cortisol were assessed biweekly from the start and over the course of treatment. The outcome groups initially differed in estradiol levels (WCR group significantly higher), although all patients had levels clearly in the subnormal range. Body composition measures revealed significant fat mass differences among the three outcome groups. Leptin levels changed over time ($P < .01$), but the NWR changed significantly

less than the other two outcome groups (P< .01). In the WCR group leptin levels normalized after approximately 4 months and a threshold was detected; an initial leptin level > 2 microgr./L increased chances for a full recovery to 75%.

For the HPG-axis hormones normal values were reached in the WCR group only. Cortisol normalized faster in the WCR group than the other outcome groups. ACTH showed no change over time in any group and all values were in the normal range. Although not completely understood this is in line with previous findings that in AN, increased central CRH, hypercortisolemia, and normal ACTH levels are found with maintenance of the circadian rhythm (Licinio et al., 1996; Gold et al., 1986). IG-F1 levels finally increased over time with the WCR group showing the highest (but still subnormal) levels.

Without normalization of weight, no normalization of hormones is to be expected. However, with a comparable and normalized weight WCR and WR groups show differences in leptin levels, fatmass and HPG-axis hormones. Rates of change between the NWR group and the other two outcome groups differ significantly for almost all hormone values. Between the WCR and WR groups significantly different rates are found for all HPG-axis hormones. These differences reflect the complicated nature of weight recovery during treatment for AN and the necessity to take body composition and changes in hormone levels, especially leptin into account.

Nurse evaluation of hyperactivity in anorexia nervosa: a comparative study

Up to 80% of patients with AN manifest elevated levels of physical activity or hyperactivity (Hebebrand et al, 2003). The exact nature of hyperactivity remains to be clarified. In addition, it is not included in the DSM IV criteria, but several authors argue that it is one of the core symptoms of AN (Hebebrand et al., 2004; Casper, 1998).

A variety of methods has been used to evaluate activity levels, mostly questionnaires but also methods such as actometry or other measurements of energy expenditure. Nurse observations have heretofore not been tested for validity and reliability. To be able to study activity levels in patients, we performed a validation study in which we compared activity ratings by nurses and through a device (Actiwatch, Cambridge Neurotechnology, Cambridge, United Kingdom).

In this study, 18 patients with anorexia nervosa under treatment in a specialized eating disorder centre simultaneously rated their own physical activity levels, used an actometer, and were observed for hyperactivity by trained nurses. We found that nurse ratings of physical activity correlated significantly with the average actometer activity score ($r= 0.61$, $p< 0.01$). Patients could not rate their own physical activity levels accurately. Nurse observation of physical activity levels of anorexia nervosa patients during treatment is a reliable and useful monitoring tool.

The impact of hyperactivity and leptin on recovery from anorexia nervosa

Using nurse observation as a method we then proceeded to look at the activity levels of the patients in the follow up cohort study. Hyperactivity has been associated with low leptin levels in the acute stage of AN and in animal models of anorexia nervosa. To further understand the importance of this correlation in AN, we investigated the relationship between hypoleptinaemia and hyperactivity in AN patients longitudinally and assessed their predictive value for recovery.

Body weight, activity levels, and serum leptin levels were assessed at the start and during treatment, up to a year. In the adolescent group, initial leptin and activity levels were correlated. This negative correlation changes over time into a positive correlation with physiological recovery. Treatment outcome in both the adolescent and the adult group could be predicted by initial BMI and leptin levels but not by activity levels. No major relationship of activity with the course of recovery was detected, suggesting that in contrast to the acute stage of the disease, leptin and activity levels during the recovery process are dissociated.

Olanzapine reduces physical activity in rats exposed to activity-based anorexia: implications for treatment of anorexia nervosa

Animal models of AN might contribute to the understanding of AN and subsequently improve treatment. Activity-based anorexia (ABA) is considered an animal model of AN and mimics food restriction and hyperactivity in rats. The ABA model consists of a combination of limited access to food (usually 1-2 hrs./day) and access to a running wheel, rats exposed to the ABA-model increase their total daily running wheel activity up to three fold and lower their

food intake, lose weight and become hypothermic and 'stressed'. Treatment with olanzapine (Zyprexa®), an atypical antipsychotic with limited extrapyramidal effects and associated with body weight gain was evaluated in the ABA model and AN patients.
Rats exposed to the ABA model were chronically infused with olanzapine (7.5 mg/kg/day) and development of ABA was studied. The patients in the follow up cohort study who received olanzapine, (5 mg) were studied in a clinical study.

Olanzapine treatment in rats exposed to the ABA model significantly reduced running wheel activity. Olanzapine also reduced starvation-induced hypothermia and decreased HPA axis activation in ABA rats. In addition, olanzapine treatment reduced activity levels of AN patients as compared to untreated AN patients in the clinical study. These data lend support to the possibility that olanzapine may be useful in treating anorexia nervosa. However, a controlled trial is necessary to demonstrate that olanzapine is efficacious.

Predictors of recovery of ovarian function during weight gain in Anorexia Nervosa

It is well known that while some AN-women regain normal menstrual cyclicity immediately following body weight normalization, for other patients it can take additional months to years until the target weight has been reached. Factors underlying these individual differences remain to be explored. We investigated the predictive value of various ovarian endocrine markers (especially AMH and inhibin B) upon initial screening for the resumption of normal menstrual cycles following weight recovery in the participants of the follow up cohort study.

Next to weight gain itself, initial ovarian endocrine markers such as FSH, inhibin B and AMH hormone were capable of predicting chances for resumption of menses in a multivariate analysis with time to recovery as the main outcome measure. This study shows that initial leptin levels do correlate with the chances for recovery of ovarian function and a threshold value could indeed be established. However, after correction for a possible association with other endocrine markers by means of multi-variate analysis, leptin disappeared as the sole or main predictor.

Changes in mood states during recovery from Anorexia Nervosa

In AN recovery is a complex issue. Physical recovery like return of menses is often not accompanied by recovery of psychological symptoms of AN such as distorted attitudes to food and appearance, depressive feelings and anxiety. In this study we investigated psychological changes during the recovery process in AN to determine how these parameters alter as a function of recovery, in patients who underwent a structured treatment program, aimed at restoration of weight, eating pattern, body image, normalisation of anorectic cognitions and family and social functioning.

Using the shortened version of the Profile Of Mood States scale (POMS) we assessed mood states weekly in the 55 of the 61 patients who participated in the follow up cohort study from the start and over the course of treatment, up to a year. At the start of the study the overall picture of the patient group is of extremely high scores on the subscales 'depression' and 'tension', high scores on the subscale 'anger', above average scores for 'fatigue' and below average for 'vigor'. All subscales showed significant changes over time, decreased levels on the subscales 'anger', 'depression' 'tension' and 'fatigue' and increased levels on the subscale 'vigor'. Effect sizes differed between the three different groups (NWR, fifteen patients; WR, eighteen patients; and WCR, twenty three patients). For the subscales 'depression' and 'tension' the WR group showed a significantly larger reduction. The 'anger' subscale showed a significant reduction in all groups, and although effect sizes differed between the outcome groups this did not reach statistical significance. On the 'fatigue' subscale a significant reduction was visible overall, with similar results for the WCR and WR outcome groups, but the NWR group showed an increase in symptoms instead of a reduction. The 'vigor' subscale showed the opposite picture: a significant positive effect over time, similar in the WCR and WR group, but less for the NWR group, reaching statistical significance. Over all physical recovery was accompanied by changes in mood states. Restoration of weight or even full physical recovery including resumption of a menstrual cycle however is not straightforward followed by a normalisation of mood. After nine months in treatment for AN, anxiety and depression symptoms scores remain in the clinical range, and would require treatment in itself.

Outcome of Anorexia Nervosa: results of a 5 year follow-up study

Approximately 5 years after the first admission we approached the participants of the original study cohort to investigate the current state of illness as well as to identify potential predictors of recovery. We contacted all 61 patients and investigated body weight and menstrual status, as well as psychological features like body attitude, psychopathology, mood states and social and occupational adjustment. Of the original sample 59% of the original participants had a healthy weight and normal menstrual status, 9.8% a healthy weight but no regular menstrual cycle and 31.1% was not recovered. None of the participants had died. Of the original 61 participants, 54 subjects cooperated on a further investigation of psychological features. Of this group, 16 still met AN criteria, whereas 12 met EDNOS criteria. No respondent met BN criteria. Almost half of the respondents still reported co-morbid problems and psychological complaints (SCL90), mainly depression, anxiety, OCD and personality disorders, and 70% scored in the clinical range of a disturbed body attitude. Co-morbidity and psychopathological complaints (SCL90) were found significantly more frequent in NR than in WCR respondents. Body attitude was also significantly more disturbed in NR than WCR respondents. At T5 respondents POMS scores showed less depression and anger but still very high tension-anxiety scores, again mood was significantly worse in NR versus WCR respondents. The WCR group scored significantly higher on subjective scores of overall happiness.

Plasma leptin levels and nurse ratings of patients hyperactivity at T1 were no predictors of treatment outcome at T5, although it must be mentioned that log leptin levels at T1 tended to correlate with treatment outcome at T5 ($p=0.06$, $\rho=0.251$, n.s.) and were together with BMI at T1 the most likely predictors of treatment outcome. In addition WCR subjects who changed into NR at T5 had higher activity levels at T1, as measured by trained nurses. So although in the first year activity levels did not correlate with outcome, they do seem to influence chances for relapse.

General discussion

The aim of this thesis was to gain more insight in the various aspects of changes occurring during the trajectory toward the first recovery from anorexia nervosa, defined as a normalized weight and resumption of menses, to identify factors that predict or influence final recovery outcome.

Recovery from AN is a complex issue with physical and psychological aspects and short term and long term goals. Our outcome data are in line with the literature (Strober et al., 1997; Fennig et al., 2002; Eckert et al., 1995, Steinhausen et al., 2002) and indicate we studied a representative sample for a tertiary referral center. Physical recovery was defined as a normalized weight and resumption of a regular menstrual cycle and this study shows it is possible to predict these two components of physical recovery in the first year of treatment. Psychological recovery from the eating disorder symptoms such as distorted body image we did not expect to occur in the first year of treatment (Strober et al., 1997) but we studied co morbid symptoms by following changes in mood states, especially anxiety and depression.

Physical recovery

Weight

In AN, restoration of physical health takes time. At the end of the first year 67% of the patients has a normalized weight and 57% of that group shows full physical recovery. The results at the end of the initial study compared with those at follow up several years' later show that weight recovery at a moderate rate in the first year (the WR group) is not a bad start: the WR group has almost disappeared at the 5 yrs FU. Of the 18 original patients in this group at T1, two-thirds move on to the weight and cycle recovered group and only 5 end up in the non recovered group at T5. Holtkamp pointed at the risk for relapse in those patients with the highest leptin levels at the moment of discharge at the end of inpatient treatment (Holtkamp et al., 2004). Our treatment schedule differs from the one described by Holtkamp and co-workers, because the length of inpatient treatment is not set as in their study but varies depending on weight gain. This means that at discharge patients have usually stabilized in

the normal weight range. We could not replicate their findings that the highest leptin levels at discharge are correlated with relapse, but it is clear that the WR group showed lower leptin levels after a year of treatment than the WCR group. Although there have been several reports on the risks of fast weight recovery, no studies sofar have compared the outcome results of patients who followed the advised rate of weight gain versus those who went at a slower pace. Further study into this subject seems worthwhile, especially with regards to early prediction of the rate of recovery in relation to recovery outcome .

Almost all hormones changed over time with weight gain, with ACTH being the only exception to this rule. If normal values were reached this occurred in the WCR group only.

The rate of change was the highest in the WCR group. Up to date there are no studies we know of that have studied changes during recovery on a longitudinal basis. Most studies compare initial findings and data gathered once recovery has been reached, our start and end findings are in line with this literature.

Menses

In this follow up cohort several factors predicted and influenced complete physical recovery in the first year. A novel finding is that the ovarian markers AMH, Inhibin B together with FSH predict which participants will regain a regular menstrual cycle with weight gain. We confirmed the findings of several authors (Kopp et al., 1997; Audi et al., 1998; Holtkamp et al., 2003) about leptin and the menstrual cycle. We found that leptin levels did predict physical recovery, but more interestingly we detected a threshold level; an initial leptin level > 2 microgr./L increased chances for a full recovery to 75%.

Activity level & olanzapin

We replicated the finding by Holtkamp et al. (2006) that hyperactivity is related to leptin in the acute stage of AN but only when we compared an age matched group, the youngest patients in this study. Activity levels however are not predictive for treatment outcome in the first year in our study sample, higher activity levels correlate with lower leptin and in that way influence chances for recovery. Interestingly, chances for a patient that shows recovery in the first year,

to relapse at 5 years are correlated with leptin and activity level at T1, a novel finding. The use of the atypical neuroleptic olanzapin in the treatment of AN has been described in several case studies (Boachie et al 2003; Malina et al 2003) Further RCT studies into the effects of olanzapin on activity levels, with leptin as a clinical marker seem very useful.

Psychological recovery

Without physical recovery there is no psychological recovery, but why in some patients these two improve on a parallel track whereas in others there does not seem to be any correlation at all and the factors that influence psychological recovery need further study. Although at 5 years FU 59% of the participants had become physically recovered and 52% did not meet criteria for an eating disorder, at closer observation many still spent a more than average amount of time and energy on thoughts concerning food or their body. Most striking are the data on the anxiety and depressive symptoms that are in line with the findings of Herpertz-Dahlmann and colleagues (2001). Although the results from recent studies show that antidepressant medication does not prevent relapse in the first year after treatment of AN (Walsh et al 2006) the question remains how to address the persisting comorbid symptoms.

Conclusions

Clinical implications

Recovery from AN obviously is a very long-term goal to be reached, with many and often seemingly unexpected obstacles on the trajectory towards health. Our current state of the art treatment can be helpful with regards to physical recovery in the first year of care if we set individual target weights sufficient for the resumption of menses. In this study, in the adolescent treatment centre target weight had been set too low at first, using the BMI 25th percentile chart. Meanwhile we have implemented individual set target weights, using growth charts. Further physical recovery after the first year does occur; in the participants of this study continued over the years reaching about 60% at 5 yr FU. With regards to psychological recovery the results of our current treatment procedures are much less predictably successful

in the first year, especially in the areas of anxieties and depression. Although further recovery occurs over the years towards the FU, this area has room for improvement. In this study at the 5 yr FU many former participants still suffered both from eating disorder related symptoms and also from anxiety or depression.

The conviction many patients express, that to stop eating (again) will mean to feel better, often meaning less emotions, does not hold. This study shows that both in the first year of recovery but also at 5 yr FU, a normalized weight leads to better scores on symptom- and moodscales and also improves (but without normalization) body attitude.

The results on the rate of weight gain in this study raise some interesting points, firstly; patients who show a moderate rate and belong to the WR group at the end of the first year of recovery have a chance of 66% of full physical recovery at FU 5 years later and thereby equal the chances of the WCR group. This suggests there is a (recognizable) subgroup that would benefit from a program aimed at slow and steady weight gain. Secondly; patients who gain weight and reach a normal body weight will not always normalize in body composition, patients in the WR group were found to have a lower percentage body fat linked to lower serum leptin levels and continued amenorrhea. At the moment clinical use of leptin as a marker should be implicated, measuring body composition with special scales is a reasonable alternative. With the use of biological markers as AMH and leptin it is possible to predict this rate and monitor physical progress, which enables us to produce more tailor made treatment programs for the first year. After the first recovery it remains difficult to predict relapse and further studies need to be done to identify risk factors.

Hyperactivity, although not a DSM IV criterion, is an important concomitant symptom to be dealt with in treatment. In the acute stage of the disease, at least in young patients, activity is linked to leptin levels and in that way influences treatment progress. This finding could not be confirmed in the adult patient group, possibly because of a more strict inpatient regime, or because activity is a developmental phenomenon that decreases with rising age. As it is clear that nurses are able to observe activity levels in patients, and also that olanzapine improves this symptom, the treatment of inpatients can be improved by paying specific attention to hyperactivity.

Psychological recovery with a focus outside the eating disorder symptoms turned out to be an area that we know surprisingly little about. The results of this study make it clear that it is important to observe in detail the anxieties and worries patients experience during and at the end of the first year and to tailor subsequent additive treatment. Do these patients remain to be treated with anti-depressants after they have physically recovered? At our FU recovery from AN is far from finished; although 60 % of the patients has reached a healthy physical condition, psychologically stability has often not been reached and both eating disorder symptoms and co morbid symptoms threaten this fragile balance.

Strengths and weaknesses

As far as we know this is the first study that describes a natural, randomly selected, albeit tertiary treatment referrals only, group of patients during their first year of treatment. Although methodologically extremely challenging, the results are clinically much more interesting and relevant. A longitudinal study in very young patients with AN is a complex enterprise. Drop out numbers become too high if one is not flexible, with missing data as a result. Balancing the two sides of this medal is only possible in a setting that is both clinically and scientifically interested. The two treatment centers that participated in this study meanwhile have merged and their diagnostic and treatment procedures have become much more uniform as a result.

Future perspectives

Studying or treating an illness that so clearly has both physical and psychological aspects, obviously requires input from both sides. Multidisciplinary research teams that preferably have both preclinical and clinical ties are a prerequisite to create research questions that can lead to better insights in the processes that trigger and maintain the many different aspects of AN. Genetic studies, animal experiments, endocrinological markers, questionnaires tracking traits and states are still necessary to develop new pharmacological agents, develop stepped care programs suited for patients with different profiles and improve the treatment and prognosis for patients with eating disorders.

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Nederlandse samenvatting

Nederlandse samenvatting

Anorexia nervosa: klinisch beeld

Anorexia nervosa (AN) is een complexe psychosomatische ziekte van onbekende oorsprong, die vooral jonge meisjes en vrouwen treft. AN wordt gekenmerkt door een afwijkend eetpatroon en gewichtsregulatie waardoor gewichtsverlies en stoornissen op endocrien gebied ontstaan die zich onder meer uiten in het wegblijven van de menstruatie. Er is sprake van een intense angst om in gewicht toe te nemen en een vertekend lichaamsbeeld. Patiënten voldoen aan de DSM-IV criteria van AN als ze niet in staat zijn om een minimaal normaal lichaamsgewicht te handhaven (BMI < 17.5 kg./m^2 voor volwassenen), een intense angst hebben om dik te worden, een gestoord lichaamsbeeld hebben en secundaire amenorroe vertonen. Er bestaan twee subtypes: een afwijkend eetpatroon, dat gedomineerd wordt door vasten in combinatie met hyperactiviteit – het restrictieve type – of door vasten afgewisseld met vreetbuien, braken en laxeren – het purgerende type.

Het klinische syndroom AN stamt van de Engelsman Gull en de Fransman Lasègue, die in de 19^e eeuw publiceerden over een ziektebeeld gekenmerkt door amenorroe, obstipatie, verlies van eetlust, uitmergeling en een verminderde vitaliteit. Hun beschrijvingen van de lichamelijke verschijnselen, de vitaliteit en overactiviteit van cachectische patiënten, hun gebrek aan ziekte-inzicht en hun herstel via psychologische beïnvloeding van het eetpatroon zijn nog steeds van toepassing.

AN gaat gepaard met hoge morbiditeit- en mortaliteitscijfers; stabiel lichamelijk herstel treedt gemiddeld na 4.7 jaar op en psychosociaal herstel pas na 6.6 jaar, recidieven treden frequent op vooral in het eerste jaar na behandeling. De prognose van AN is matig, slechts 50% van de patiënten bereikt volledig herstel en 20% blijft chronisch ziek. AN heeft de hoogste mortaliteitscijfers van de psychiatrische ziektebeelden: 10-15%.

Men veronderstelt dat AN een multifactoriële etiologie heeft, een combinatie van een genetische kwetsbaarheid, uitgelokt door omgevingsinvloeden. Uit genetische studies is gebleken dat zich op chromosoom 1, 2 en 13 interessante regio's bevinden en associatie studies hebben de betrokkenheid aangetoond van bepaalde genen zoals verschillende 5-HT receptoren, AGRP en BDNF.

Er bestaat nog nauwelijks evidence based behandeling voor AN, de beste behandelvormen zijn gebaseerd op de Amerikaanse en recent ook de Nederlandse richtlijnen, met aanpassingen voor een jongere leeftijdsgroep, vooral in de vorm van betrokkenheid van het gezin bij de behandeling.

Longitudinaal onderzoek

Dit proefschrift is gebaseerd op een follow up studie in een unieke onderzoeksgroep met deelnemers die jonger waren dan in de meeste studies en die tweewekelijks gevolgd werden gedurende het eerste jaar van hun behandeling en niet zoals meestal een begin en een eindmeting. De studiedeelnemers waren de opeenvolgend verwezen patiënten van twee geografisch nabijgelegen gespecialiseerde behandelcentra, één voor adolescenten (op de afdeling Kinder- & Jeugdpsychiatrie van het Universitair Medisch Centrum Utrecht) en één voor volwassenen (Rintveld, centrum Eetstoornissen, onderdeel van Altrecht GGZ).

De groep bestond uit 61 vrouwelijke AN patiënten met een gemiddelde leeftijd van 18.2 ± 3.1 (SD)jaar, 15.9 ± 1.2 jaar voor de adolescente groep en 20.6 ± 2.9 jaar voor de volwassen groep. Ze namen deel aan de studie tussen februari 2000 en januari 2003. Na de diagnostiekfase namen alle patiënten deel aan een gestructureerd behandelprogramma, gericht op gewichtsherstel, normalisatie van eetpatroon, lichaamsbeeld, anorectische cognities en gezins- en sociaal functioneren. De studie was vooral gericht op psychoneuroendocrinologische veranderingen gedurende de behandeling; veranderingen in lichaamsgewicht en –samenstelling, in hormonen, activiteitsniveau en stemming werden zorgvuldig geobserveerd en gemeten. Na een follow up periode van maximaal een jaar werden de patienten in 3 groepen gedeeld, aan de hand van hun klinische situatie: geen gewichtsherstel van betekenis (NWR- no weight recovery-, 19 patiënten), gewichtsherstel zonder terugkeer van de menstruatie (WR – weight recovery-, 18 patiënten) en gewicht- en hormonaal herstel (WCR – weight & cycle recovery-, 24 patiënten). Bij de start van de studie waren er geen significante verschillen tussen de patiënten wat betreft start of premorbide gewicht, duur van de ziekte (gedefinieerd als duur van de amenorroe), studiedeelname duur, activiteitsniveau of type AN (restrictief of purgerend) noch voorgeschreven medicatie. Wel verschilden de

groepen van elkaar qua leeftijd, wat samenviel met de kliniek waar men werd behandeld. Het herstel percentage van de adolescentenkliniek was 16% tegen 63% in de volwassen groep. De studieduur moest minimaal 3 maanden bedragen en deelname eindigde na 3 regelmatige menstruaties of een maximale duur van 12 maanden. Van iedereen was de menstruatie status bij 12 maanden bekend. Voortijdige studie beëindiging hing samen met problemen met bloedafname of doordat een patiënt de behandeling verliet. Uiteindelijk werden daardoor de data van de eerste 9 maanden gebruikt, daarna was het aantal nog deelnemende patiënten te klein, vooral in de herstellende groep.

De patiënten gedroegen zich met betrekking tot de behandeling conform de verwachtingen uit de literatuur: sommigen verbeterden sterk, anderen veel minder en een derde groep niet of nauwelijks. Het merendeel van de deelnemende patiënten behoefde intensieve, klinische behandeling, veelal gevolgd door dagbehandeling en/of poliklinische nazorg.

Ongeveer 5 jaar na de start van de behandeling namen de patiënten deel aan een follow up studie, om na te gaan hoe het hen was vergaan.

Veranderingen in lichaamssamenstelling en hormonen gedurende het herstel van Anorexia Nervosa

In hoofdstuk 2 worden de veranderingen tijdens de behandeling in lichaamssamenstelling en hormonen betrokken bij honger en verzadiging, menstruatie cyclus en stress reactie gedurende 9 maanden behandeling beschreven. Bij AN leidt het beperken van de voedselopname tot gewichtsverlies en daarmee tot een scala aan veranderingen in centrale en perifere hormonale feedback systemen en daarbij betrokken organen. AN patiënten reageren in veel opzichten fysiologisch op vermagering maar vertonen ook een aantal ongewone reacties.

Lichaamsgewicht en –samenstelling werden bepaald met behulp van een TANITA® weegschaal. Leptine, FSH, LH, oestradiol, progesteron, testosteron, ACTH, insulin-like growth factor-1 (IGF-1) en cortisol werden tweewekelijks gemeten vanaf de start van de studie. Aan het begin van de studie werd een significant doch niet klinisch relevant verschil geobserveerd in oestradiolniveau. De lichaamssamenstelling ging gedurende de studie verschillen tussen

de drie groepen, in het bijzonder met betrekking tot het vetpercentage. Leptine niveaus veranderden gedurende de behandeling in alle groepen maar significant minder in de NWR groep en alleen in de WCR groep bereikten de leptine waarden een normaal niveau na circa 4 maanden. Ook werd een drempel waarde vastgesteld, bij een start waarde >2 microgram/L stegen de kansen op herstel tot 75%.

Ook de hormonen betrokken bij de menstruele cyclus (de Hypothalamus-Hypofyse-Gonadale as, HHG-as) normaliseerden alleen in de WCR groep.

Cortisol waarden normaliseerden sneller in de WCR groep dan in de overige groepen. ACTH waarden vertoonden vertoonden een normaal niveau en geen enkele verandering tijdens de behandeling. De verklaring hiervoor is niet helemaal duidelijk maar onze bevindingen komen overeen met de literatuur, die aantoont dat bij een verhoogd centraal CRH en verhoogde cortisol waarden normale ACTH niveaus worden gevonden met behoud van circadiane fluctuatie.Tot slot stegen de IG-F1 waarden gedurende de behandeling met de hoogste (maar nog steeds suboptimale) waarden in de WCR groep.

Zonder herstel van gewicht is geen normalisatie van hormonaal functioneren te verwachten. Opmerkelijk was echter dat de WR en WCR groepen bij een gelijk gewicht verschilden in leptine, vetmassa en HHG-as hormonen. De snelheid van verandering was voor de NWR groep in vrijwel alle gevallen lager, en verschilde voor de HHG-as hormonen ook tussen de WR en WCR groep. Deze verschillen tonen aan dat gewichtsherstel complex van aard is in AN en dat de afwijkingen in lichaamsamenstelling en daaraan gekoppeld veranderingen in hormonen, met name leptine, bij de behandeling dienen te worden betrokken.

Verpleegkundige evaluatie van hyperactiviteit in anorexia nervosa: een vergelijkende studie

Tot 80% van de AN patiënten vertoont verhoogde fysieke activiteitsniveaus. De verklaring hiervoor is niet volledig duidelijk. Hoewel dit symptoom geen onderdeel is van de DSM-IV criteria wordt het door verschillende auteurs beschouwd als een kernsymptoom van AN. Omdat in verschillende eerdere studies leptine niveaus en activiteit met elkaar leken samen te hangen, besloten we dat activiteit een belangrijke observatie maat was bij het bestuderen

van longitudinale veranderingen. Activiteit bij AN patiënten is op allerlei manieren gemeten, meestal met behulp van vragenlijsten, soms ook met actometrie of andere methoden om het energie verbruik te meten. Observatie door verpleegkundigen is nog niet eerder getest op validiteit en betrouwbaarheid en om dergelijke observaties te kunnen gebruiken bij het beoordelen van patiënten werd een validatie-studie verricht waarbij verpleegkundige observaties werden vergeleken met de gouden standaard: actometrie. 18 patiënten met AN, namen tijdens hun behandeling deel aan de studie. Gedurende drie werkdagen droegen ze een Actiwatch, werden geobserveerd door twee verpleegkundigen en beoordeelden zelf hun activiteitsniveau. De verpleegkundige observaties bleken te correleren met de Actiwatch metingen, terwijl de patiënten niet in staat bleken hun eigen activiteitsniveau goed te beoordelen.

De invloed van hyperactiviteit en leptine op herstel van anorexia nervosa

Met behulp van verpleegkundige observatie werden vervolgens data verzameld bij de deelnemers aan de longitudinale studie. In de acute fase van AN en ook in diermodellen is een verband gevonden tussen lage leptine niveaus en hyperactiviteit. Om deze relatie te vervolgen gedurende herstel werden activiteitsniveaus, leptine en lichaamsgewicht bestudeerd vanaf de start van de studie tot maximaal een jaar later. In de adolescenten groep maar niet in de volwassenen groep werd de eerder gevonden correlatie tussen leptine en activiteit bevestigd. Deze negatieve correlatie veranderde bij de WCR groep na 16 weken in een positieve correlatie. Startgewicht en -leptine voorspelden het herstelverloop, activiteit deed dat echter niet. Leptine en activiteit dissociëren gedurende het herstel.

Olanzapine vermindert fysieke activiteit in ratten blootgesteld aan het activity-based anorexia model: implicaties voor de behandeling van anorexia nervosa

Dier modellen van AN dragen bij aan verdere inzage in de achtergrond en behandeling. Het activity-based anorexia (ABA-) model wordt beschouwd als een diermodel voor AN omdat het voedselbeperking en hyperactiviteit nabootst bij ratten. Het ABA-model bestaat uit een

beperking van de toegang tot voedsel tot 1-2 uur/dag in combinatie met het aanbieden van een loopwiel. In een dergelijke conditie geplaatst laten ratten een drievoudige activiteitstoename zien, verliezen gewicht en vertonen hypothermie en stress. Behandeling met Olanzapine, een atypisch antipsychoticum met beperkte extrapiramidale bijwerkingen en een associatie met gewichtstoename werd geëvalueerd in ratten en in mensen. Ratten in het ABA-model kregen via een infuus Olanzapine toegediend (7.5 mg./kg./dag). Hun gedrag werd vergeleken met patiënten in de longitudinale studie die Olanzapine kregen.

Bij de ratten verminderde Olanzapine het lopen in het loopwiel, de hypothermie en de stress- as activatie. Ook bleek olanzapine het activiteitsniveau in mensen te verminderen, een gecontroleerde studie dient dan ook deze resultaten te vervolgen en het gebruik van Olanzapine te onderschrijven.

Voorspellers van herstel van ovariële functie bij gewichtstoename in Anorexia Nervosa

Het is bekend dat sommige vrouwen bij gewichtsherstel een normale menstruatie terugkrijgen, terwijl dat bij andere patiënten nog maanden tot jaren op zich kan laten wachten. De factoren die hieraan ten grondslag liggen zijn niet volledig opgehelderd. Bij de deelnemers aan de longitudinale studie werd gekeken naar de voorspellende waarde van de start waarde van een aantal ovariële markers, in het bijzonder het Anti-Műllerian hormoon (AMH) en inhibine-B. Met behulp van gewichtsstijging in combinatie met de startwaarden van FSH, AMH, en inhibine-B bleek het goed mogelijk de kans op terugkeer van de menstruatie te voorspellen. De voorspellende waarde bleek hierbij groter dan die van leptine.

Verandering in stemming gedurende herstel van Anorexia Nervosa

Bij het herstellen van AN worden lichamelijk herstel en de terugkeer van de menstruatie vaak niet vanzelfsprekend gevolgd door een verbetering van het algemen welbevinden. Veel patiënten blijven ernstige angst- en stemmingsklachten vertonen, naast restanten van eetstoornispathologie, waarmee de kans op terugval toeneemt.

Met behulp van de Profile of Mood States (POMS) scoorden de deelnemers aan de studie zichzelf wekelijks op gevoelens van somberheid, spanning, vermoeidheid, vitaliteit en boosheid. Bij aanvang van de studie scoorden de deelnemers zeer hoog op somberheid en spanning, hoog op boosheid, bovengemiddeld op vermoeidheid en laag op vitaliteit.

Tijdens de studie veranderden de scores: somberheid en spanning namen af, vooral in de WR groep, vermoeidheid nam af in de WR en WCR groep maar toe in de NWR groep terwijl vitaliteit het tegengestelde liet zien. Boosheid verminderde ook maar niet significant. Fysiek herstel leidde tot verandering van algemeen welbevinden, maar niet altijd en bij iedereen. Ook na 9 maanden studie scoorden veel patiënten nog in de klinische range angst- en stemmingsklachten, klachten die absoluut nog behandeling behoeven.

Verloop van Anorexia Nervosa: resultaten van een 5 jaar follow-up studie

Ongeveer 5 jaar na opname werden alle deelnemers aan de studie opnieuw opgezocht om te beoordelen hoe het met hen ging. Het lukte om alle 61 oorspronkelijke patiënten te traceren. 59% bleek een normaal gewicht en een regelmatige menstruele cyclus te hebben, 9.8% viel in de WR categorie en 31 % in de NWR groep. Niemand was overleden. 16 % van de deelnemers voldeed nog steeds aan de DSM-IV criteria voor AN en 12% aan de criteria voor een eetstoornis NAO, BN kwam niet voor.

Bijna de helft van de deelnemers kampte nog met psychische klachten, meestal depressie en angst, en 70% scoorde nog steeds zeer hoog op een vragenlijst omtrent lichaamsbeleving. De POMS-scores waren wel verbeterd, vooral in de WCR groep.

We vonden geen voorspellers van de uitkomst behoudens recidive in de eerste tijd na de behandeling. Wel werd geconstateerd dat de patiënten die 5 jaar na dato van de WCR groep naar de NWR groep waren gegaan, een hoger initieel activiteitsniveau vertoonden, maar de groep die hiertoe behoorde was te klein om definitieve uitspraken te doen.

Conclusies

Herstel van AN is een langdurig en complex proces met nog altijd veel onbekende variabelen. Herstel duurt lang, en een gematigd resultaat (de WR groep) aan het eind van het eerste jaar lijkt geen al te slechte start: juist de midden groep verdwijnt na 5 jaar om voor een belangrijk deel weer op te duiken in de WCR categorie.

Hormonaal herstel met betrekking tot menstruatie blijkt te voorspellen, de gevonden resultaten behoeven verdere uitwerking voor klinisch gebruik.

Hoge activiteitsniveaus zijn goed in te schatten door verpleegkundigen en lijken in de acute fase een belangrijke biologische basis te bevatten. Olanzapine kan dit symptoom beïnvloeden, maar clinical trials zijn nodig om dit te bevestigen.

Zonder fysiek herstel geen psychologisch herstel, dat is duidelijk, maar het omgekeerde is minder waar: fysiek herstel wordt zeker niet altijd vanzelf gevolgd door psychologisch herstel. 5 jaar na dato levert een groot deel van de studie deelnemers nog altijd strijd zowel tegen een vertekend gevoel over het eigen lichaam als ook tegen somberheid en angstklachten. Aanvullende behandeling is voor hen een noodzaak. Afvallen om de nare gevoelens kwijt te raken, een overtuiging die patiënten nog wel eens hebben, is geen oplossing blijkt uit deze studie!

Dankwoord

Dankwoord

Na een betrekkelijk groot aantal jaren (ik dacht 7 à 8, ik ken echter mensen die overtuigd zijn van het dubbele) is dit proefschrift af.
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Dat ik uiteindelijk met een boekje in het Academiegebouw beland heeft alles te maken met mijn promotor, Prof. Herman van Engeland.
Vanaf 1990, toen ik als arts assistent bij hem in opleiding ging, is hij er in geslaagd mij beslissingen te laten nemen waar ik uit mijzelf nooit zo op zou zijn gekomen. Van academische kinderpsychiater in wording tot buitenlandse stages, optredens in het engels en duits, een zorgprogramma en uiteindelijke een kleine volksverhuizing. En dat alles met behoud van relatie en veel waardering voor de goede kanten daaraan.

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Curriculum Vitae

CURRICULUM VITAE

Annemarie van Elburg was born in Rotterdam on June 1st 1958. She graduated in medicine in 1984 at the Free University of Amsterdam. After spending a year at 'The Hospital for Sick Children, Great Ormond Street' in London with prof. Graham, she specialized in adult psychiatry at Vijverdal in Maastricht (Prof. Richartz) and subsequently in child & adolescent psychiatry at the Department of Child & Adolescent Psychiatry of the University Medical Center Utrecht (Prof van Engeland). Part of her training was done in San Francisco, USA, at the Langley Porter Institute of UCSF with Prof. Elliott.

After finishing her residency in 1992 she continued to work at the same department, where in the mid-nineties she developed the eating disorder program for children and adolescents together with Mariette Robbe and Marjolein Rijken.

Work on this thesis started in 1999. The eating disorders research group subsequently was founded in collaboration with the Rudolf Magnus Institute for Neurosciences. Together with Prof. Roger Adan, Martien Kas, Jacquelien Hillebrand and Mariken de Krom genetic work began, later evolving into translational research, combining insights from animal work and patients.

In 2005 the eating disorder program moved to Rintveld, centre for eating disorders at Altrecht Mental Health Institute, Zeist; where she is medical director.

She is advisor to the patients organization SABN, and has been involved in national and international collaboration around research, training and clinical needs for patients with eating disorders.

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