ENERGY METABOLISM AND BODY COMPOSITION FOLLOWING RECOVERY FROM ANOREXIA NERVOSA

by

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ABSTRACT OF THE DISSERTATION

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Anorexia Nervosa (AN) affects millions of women however, little is known about the long-term effect of AN on body composition and metabolism in those who recover from AN. In short-term recovery from AN women have increased central adiposity, increased cortisol levels, and low resting energy expenditure compared to women without a history of an eating disorder. However, it is still unknown if these differences in body composition, cortisol, and energy metabolism persist in long-term recovery from AN.

The long-term effect of AN on of body composition, cortisol, and energy metabolism has not been examined. In general, undernutrition has been associated with an increased risk for increased cortisol levels, altered energy metabolism, and increased central adiposity. However, it is possible that there may be differences in the effects of undernutrition occurring in adolescents or adults. Therefore, the objective of this study was to determine the relationship between a history of AN, followed by at least 2 years of recovery (RAN), on body

composition, cortisol levels, and energy metabolism. Using dual energy x-ray absorptiometry, fat mass and body fat distribution did not differ between RAN and control participants. Salivary cortisol samples obtained at baseline and following a mental stress test did not differ between the two groups. While there were no differences in resting energy expenditure, even when adjusted for body composition, the respiratory quotient of RAN women was significantly lower, corresponding to a higher rate of fat oxidation, compared to C women. Based on the results of this study, it may be concluded that RAN women exhibit differences in substrate metabolism, without presenting differences in body composition, cortisol levels, and resting energy expenditure. Therefore time following undernutrition has an influence on body fat distribution, cortisol, and energy metabolism. Furthermore, RAN women should not be at risk for central adiposity, differing from those who experience undernutrition early in life.

ABBREVIATION LIST

ACTH -Adrenocorticotropic Hormone

AN-Anorexia Nervosa

AUC- Area Under the Curve

AGA- Average for Gestational Age

BMI- Body Mass Index

BSQ- Body Shape Questionnaire

C-Control

DSM- *Diagnostic and Statistical Manual of Mental Disorders*

DXA-Dual Energy X-Ray Absorptiometry

ED-Eating Disorder

EDNOS-Eating Disorder Not Otherwise Specified

FM-Fat Mass

FQ- Food Quotient

GLM- General linear model repeated

HPA-Hypothalamic-Pituitary-Adrenal

IC- Indirect Calorimetry

ICD-10- International Statistical Classification of Diseases and Related Health Problems 10

LBM- Lean Body Mass

LPL- Lipoprotein Lipase

NPRQ- Non-Protein Respiratory Quotient

PSPS-Perfectionistic Self-Presentation Scale

RAN-Persons in long-term recovery from Anorexia Nervosa

RQ-Respiratory Quotient

REE-Resting Energy Expenditure

REEadj- Resting Energy Expenditure Adjusted for Lean Body Mass

REE: LBM- Resting Energy Expenditure per KG of Lean Body Mass

SGA-Small for Gestational Age

TSST- Trier Social Stress Test

TrFM- Truncal Fat Mass

AdjTrFM- Truncal Fat Mass Adjusted for Fat Mass

VAT- Visceral Adipose Tissue

DEDICATION

To my grandfather for helping me to achieve all that I set my heart and mind to, being supportive in all that I have pursued and always remaining by my side. Seeing you at my defense meant the world to me. I could not have done this without you; thank you and I love you!

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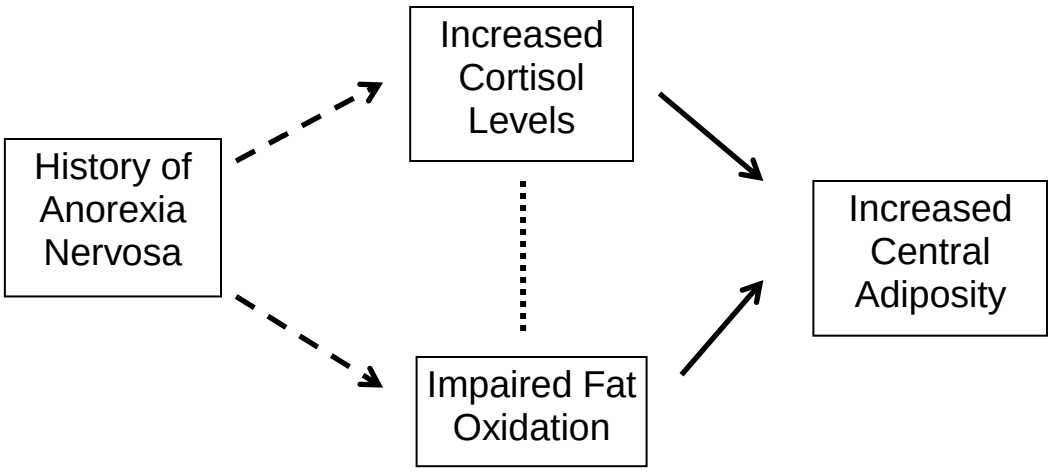
1.1 Statement of the problem

Anorexia Nervosa (AN) is a severe psychological disorder characterized by severe self-starvation and affects approximately 1% of the female population in Westernized society (1). Short-term recovery from AN is associated with increased central adiposity (2-4) and cortisol levels higher than women without a history of an eating disorder (2,4), while the long-term effects of AN on terms of body composition, cortisol and energy metabolism are unknown. Similar to women in short-term recovery from AN, persons who experienced undernutrition in early childhood have been shown to have increased central adiposity (5,6) and increased cortisol levels (7,8). In addition, undernutrition early in life has been associated with impaired substrate oxidation (9,10) and increased risk for chronic diseases (5,6,11-13). Still, data are lacking on the long-term effects of starvation following growth in adolescents and young adults. Therefore research is needed to determine the long-term effects of AN.

The objective of this study was to examine the long-term effects on AN on body composition, cortisol levels, cortisol response to stress and energy metabolism. We hypothesized that persons who suffered from AN may have high central adiposity, high cortisol levels in a baseline and stressed state, high cortisol response to stress and high respiratory quotient. Each of these factors could result in increased FM and central fat deposition **(Figure 1-1)**.

Figure 1-1 Possible Relationship between Anorexia Nervosa, Altered Body Composition, Altered Metabolism, Cortisol and Chronic Disease



2 LITERATURE REVIEW

2.1 Anorexia Nervosa Overview

Anorexia Nervosa (AN), a severe psychological disorder, is considered one of deadliest psychological disorders (14-19) and is an Axis 1 disorder within *The Diagnostic and Statistical Manual of Mental Disorders* (DSM) *IV* (20)***.*** DSM-IV has established the following diagnostic criteria for AN:

> "A. Refusal to maintain body weight at or above a minimally normal weight for age and height (e.g., weight loss leading to maintenance of body weight less than 85% of that expected; or failure to make expected weight gain during period of growth, leading to body weight less than 85% of that expected).
>
> B. Intense fear of gaining weight or becoming fat, even though underweight.
>
> C. Disturbance in the way in which one's body weight or shape is experienced, undue influence of body weight or shape on self-evaluation, or denial of the seriousness of the current low body weight.
>
> D. In postmenarcheal females, amenorrhea, i.e., the absence of at least three consecutive menstrual cycles. (A woman is considered to have amenorrhea if her periods occur only following hormone, e.g., estrogen, administration)" (20)***.***

The DSM-IV has further divided AN into two types as follows:

> **"Restricting Type:** during the current episode of Anorexia Nervosa, the person has not regularly engaged in binge-eating or purging behavior (i.e., self-induced vomiting or the misuse of laxatives, diuretics, or enemas)
>
> **Binge-Eating/Purging Type:** during the current episode of Anorexia Nervosa, the person has regularly engaged in binge-eating or purging behavior (i.e., self-induced vomiting or the misuse of laxatives, diuretics, or enemas)" (20)*.*

2.1.1 Anorexia Nervosa- Prevalence and Incidence Rates

AN is a serious disorder with severe and potentially deadly consequences and affects a significant number of persons each year. Overall, the prevalence of AN is considered to be between 0.5%-1% of the female population in Westernized societies (1,21-23) and 0.02% to 0.1% of the total population (21,24-26).

DSM-V is on the horizon and could influence the prevalence of AN in the coming years as the development of DSM-V may change that diagnostic criteria for AN (27). For example, it has been proposed to remove the amenorrhea requirement from the AN diagnostic criteria (27). Thus, among women born after 1945, the prevalence of AN which has been reported to be 0.88% using the full DSM-IV criteria for AN, would be as high as 1.6% using a definition of AN that included all components of the DSM-IV criteria for AN except amenorrhea (21). In addition, slightly less than 0.4% of Portuguese females in grades 9-12 (aged 12-23) have AN, however, a much higher percentage were classified as having Eating Disorder Not Otherwise Specified (EDNOS) (28). The EDNOS category of DSM-IV diagnosis may contain a number of individuals who have many of the core pathological criteria for AN while not meeting one of the diagnostic criteria (often amenorrhea) (29-31).

While AN does not seem to increase with urbanization (32), when looking at the incidence of AN per 100,000 person-years, there has been an increase in the incidence of AN in the female population between the ages of 10-19 and 15-24 since 1935 (33). In the United States and Europe, most of the increased

incidence in AN occurred prior to 1970 (26,34,35). Retrospectively applying a definition of AN similar to that of DSM-III revised, the incidence of AN was 0.38 in 1956-1958 and increased to 1.12 by 1973 (35). Between 1973 and 1993, the incidence of AN remained relatively stable and was estimated to be 1.17 in 1993 (35). This observation is consistent with other studies that have also reported a relatively stable incidence since the 1970's or 1980's (26,34-36). Overall, the incidence of AN has been relatively stable at least since the 1970's.

While the incidence of AN may be relatively stable, more people may be seeking treatment for AN. For example, the number of new patients being seen at hospitals or similar treatment facilities for treatment of AN has increased by 5.3% between 1965 and 1991 (37). In Singapore, the number of persons seeking treatment for AN has more than quadrupled since 1998 (38). Thus, a small, but significant number of people, especially young women in Westernized society, are experiencing AN and seeking treatment for this disorder.

2.1.2 Anorexia Nervosa- Costs

It is important to study AN since AN affects a significant number of treatment-seeking individuals and has both short and long-term medical costs. While the prevalence of AN is approximately 1% of the female population (1), the prevalence of persons with AN receiving treatment at hospitals on an in- or out-patient basis is higher, 5.7% as outpatient (39). The discrepancy in the prevalence and number of people seeking treatment for AN could be due to the severe consequences of AN and the fact that AN is quite expensive to treat in its acute form (40-42). Most hospitalizations for eating disorders (EDs) are for AN

(43). It has been estimated that for all EDs the length of hospitalization is approximately 2.5 weeks with a cost of over $10,000 (43). One study has reported daily treatment costs of close to $1,000 with an average treatment length of almost 3 months (40). In Germany, a study estimated the overall cost of each case of AN in terms of treatment and lost work and productivity to be almost 200,000,000 Euros (42).

A significant number of people using the mental health system have AN, making it important to develop effective evidence-based treatment for AN. In addition, health professionals need to have adequate knowledge of health risks in this population. Hall et al. reported that 10% of persons with AN require hospital treatment in an intensive care unit (44) and AN is associated with health complications such as osteoporosis, hypercholesteremia, gastrointestinal, liver and cardiovascular sickness (14-17,45-57). For example, low HDL (49) and high cholesterol (48,50) have been shown in short-term recovery from AN. Furthermore, increases in interleukin-6 and Apo-B have been shown in acute AN (58,59) and could be associated with long-term health risks. Even when in recovery from an ED, it has been reported that persons with a history of an ED utilize health-care systems more than persons without a history of EDs (41,60,61). This poses additional costs to society. Further, it illustrates the importance of health professionals having knowledge of long-term health risks associated with AN, since people with a history of AN are likely to use the health care system. Therefore, research is needed to elucidate possible long-term health risks associated with AN. Data presented here can be used in conjunction

with other research to improve both psychological and medical treatment of this disorder in order to minimize both the health and financial costs.

2.1.3 Anorexia Nervosa- Outcome

Despite AN being one of the deadliest psychological disorders(14-19), long-term survival and full or partial recovery from AN is high (62-67). It has been shown that fewer adolescents who have been diagnosed with AN are dying as a result of AN (62). Between 1977 and 1982, 4.4% of adolescents with AN died as a result of AN compared to 1.3% between 1987 and 1991(62). Of persons who experienced AN as an adolescent, over 90% are still alive 30 years after being diagnosed with AN (63). In addition, in a Swedish only 1.2% of persons were deceased 9-14 years following hospitalization for AN (64). In general, less people are dying as a result of AN making it important to know about the long-term effects of this disorder.

Not only is the number of people dying from AN decreasing, but the estimated recovery rates for adolescents with AN ranges from 57-94% (68). For example, one study reported a 67% clinical recovery rate from AN within 5 years (69). People not only recover from AN, but many who suffered from AN have good or intermediate long-term outcomes (64-67). Only 8.7% of the 98.8% of survivors still had psychological problems that required hospital treatment 9-14 years following AN-related hospitalizations (64). Approximately 12 years following diagnosis of AN, 90% of people no longer met full diagnostic criteria for AN (65) and over 50% no longer met DSM-IV criteria for any ED (66). In this study, over 50% had good or intermediate outcomes at follow-up (66). In another

investigation, over 70% of persons hospitalized for AN no longer met diagnostic criteria for AN 21 years later (67). These individuals achieved a full or partial recovery and were deemed to have a good or intermediate outcome (67). Thirty years after being diagnosed with AN, recovery rates range from approximately 57% to 95% (70). Hence, the majority of people with AN survive and many will achieve a good or intermediate long-term psychological outcome. Surprisingly, however, little is known about the long-term physiological effects of AN.

2.1.4 Anorexia Nervosa- Why Study Long-Term Health Risks?

It has been suggested that certain health parameters, such as cholesterol, should be monitored long-term as these parameters do not correct immediately following AN and could be associated with long-term health problems (48). Further, it has been shown that shortly after weight regain, compared to women without a history of an ED, women with a history of AN have increased percent truncal fat mass (TrFM) (2-4,71,72) and increased cortisol values (2,4), both associated with increased risk for chronic diseases (73-83). If percent TrFM and cortisol levels remain higher in individuals in long-term recovery from AN compared to healthy individuals without a history of an ED, it could result in poorer long-term health outcomes and increased medical costs. Knowing this information could help medical professionals best treat this group in the short-term and long-term. Research presented here provides information about known risk factors for increased central fat gain which is a risk factor for chronic diseases. Our research explores if these known risk factors are associated with long-term recovery from AN.

2.2 Caloric Restriction Models

Much of what is known about the effects of caloric restriction comes from the Minnesota Starvation Study (84), studies of undernourished children (5) and the CALERIE study (85). However, current knowledge of long-term effects of undernutrition is still limited. While each of these studies is able to provide important information about weight loss, an important area of research not included is the study of energy restriction, in adolescents and young adults. For example, the Minnesota Starvation Study was conducted solely on males and it is possible that gender differences exist in response to starvation. In addition, methodological advancements since the Minnesota Starvation Study allow for more refined measurements and observations. Participants in the CALERIE study were overweight at the start of the study and did not have a body mass index (BMI) below 17.5. Persons born small for gestational age and stunted children experienced undernutrition prior to the start of puberty. The information provided by each of these groups provides a base of knowledge regarding the effects of caloric restriction, but more information is needed on the long-term effects of undernutrition following puberty. Therefore, studying a group of individuals in long-term recovery from AN can provide much needed information on long-term effects of starvation, information that can be applied to other populations that experience starvation later in life.

2.2.1 The Minnesota Starvation Study

The Minnesota Starvation Study conducted during World War II and involved the systematic weight loss of 25% of total body weight over a 24 week

period (84). Using hydrodensitrometry, it was shown that during weight regain, fat mass (FM) was gained more quickly than lean body mass (LBM) and total FM was higher following the study than prior to the study (86). Fat, protein and carbohydrate metabolism did not change during the period of starvation compared to the control period (84). Hence, after weight re-gain following starvation men have higher FM than prior to starvation. However, information is lacking on the long-term effects of starvation on body fat distribution and the effects of starvation on body composition in women.

2.2.2. Persons Born Small for Gestational Age

Another group of people that experienced undernutrition are persons born SGA. It has been reported that persons born SGA are at increased risk for chronic diseases (5,6,11-13). Previous studies have shown that children who experience chronic undernutrition in utero and are born SGA have increased central adiposity (5,6), a risk factor for chronic diseases. Further, researchers are starting to link being born SGA to elevated baseline cortisol levels and stressed state cortisol levels (7,8), a known risk factor for central adiposity (73,74,77), thereby increasing their risk for chronic diseases (87-89). Further studies are needed to link increased cortisol levels, increased central adiposity, and increased risk for chronic diseases in this and other populations that have experienced undernutrition.

2.2.3 CALERIE Study

The objective of the CALERIE study was to determine the effects of caloric restriction on metabolism and body composition in overweight individuals

(85). Participants voluntarily engaged in caloric restriction for a six month period of time (85). It was found that participants lost 10% of body weight and 24% of total body fat, but body fat distribution remained unchanged (85). While this study provides some information about the effects of caloric restriction in adults, it must be noted that this group did not enter into a starvation state and participants maintained a healthy or overweight BMI (85). Furthermore, only the short-term results of this intervention are known and further studies regarding the long-term effects of starvation following growth and development are needed.

2.3 Body Composition

Several groups have studied body composition in women during and shortly following weight regain from AN (2-4,90-92). These studies have varied in the criteria used to determine weight regain and may or may not have included a short period of weight maintenance prior to conducting the final measurements. In AN, FM is lower than control participants (72,93,94), while in weight restoration from AN, persons gain more FM than LBM (2-4,90-92) and TrFM exceeds that of women with a history of an ED (4). Further studies are needed to determine if differences in body fat distribution persist in long-term recovery from AN.

2.3.1 Initial Changes in Body Fat Following Anorexia Nervosa

In initial weight recovery from AN, more FM than LBM is gained (2-4,90-92), but percent FM does not exceed that of women without a history of an ED (2-4,71,72). One study reported that between 4 months and 3.3 years after an initial visit for AN, women regained between 1 kg and 13.7 kg of body weight (71). At the time of follow-up, only 6 of the 21 participants included in the study

had begun menstruating and almost 50% of the participants were purging or engaging in other compensatory behaviors (71). At follow-up, in the same study, it was reported that control participants had significantly more FM than the AN participants both at intake and compared to the AN participants at the time of the final measurement (71). In another study, 9 months following initial measurement, percent FM remained lower in AN than control participants, but the AN group was still only at 81% of ideal body weight (2). However, it has also been shown that in short-term weight recovery, persons with a history of AN have a percent FM similar to healthy, control women (3,4). Therefore, further research is needed to determine the long-term effects of starvation on FM. Furthermore, since central adiposity has been associated with increased risk of chronic diseases (87-89,95), it is important to study body fat distribution in women in recovery from AN.

2.3.2 Body Fat Distribution

For those in recover from AN, increased central adiposity is evident in the short-term following weight regain from AN (2-4). Using DXA, after re-feeding and a 2 month period of weight stabilization, defined as weight fluctuations less than 1 kg, the percent FM of the re-fed AN women was not different than control women (3). However, using anthropometrics, it was shown that the re-fed participants had significantly higher bicep and abdominal skin-fold thickness and had a significantly increased waist-to-hip ratio compared to control participants (3). Thus, despite the similar percent body fat the re-fed participants had greater central adiposity.

Other studies have also studied body fat distribution in re-feeding or in persons in short-term recovery from AN. For example, 9 months following an initial AN diagnosis, AN participants were at 81% of their ideal body weight (2). However, the AN group had a lower percent extremity fat and a higher ratio of percent trunk to percent extremity fat compared to the control group (2). While the participants were still not at a healthy body weight, it was concluded that of the FM gained, a high proportion was central fat. In another study, shortly after weight restoration (approximately 10 weeks after initial visit), the percent FM was similar to that of control participants (4). However, the weight restored group had significantly less extremity fat and significantly more TrFM compared to control participants (4).

In summary, shortly following weight regain from AN, FM is gained more quickly than LBM (2-4,90-92). While percent FM does not exceed that of control participants (2-4,71,72), percent central FM is higher in short-term recovering women than women without a history of an ED (2-4,71,72). Thus far, it appears that only one study has measured body fat distribution after two years of recovery following AN (96) and no differences in body fat distribution were found between recovering and control women (96). Still, there is a lack of information regarding body composition in long-term weight recovery from AN. This dissertation is able to contribute to the literature by providing detailed data on body composition and body fat distribution following long-term recovery from AN, information that is useful both psychologically and physiologically. Psychologically, the gain of TrFM is especially emotionally distressing to persons

suffering from AN (97). Results from this study could be used by clinicians to inform clients that about what can be expected in terms of long-term body fat distribution. Physiologically, increased central adiposity is associated with increased risk of chronic diseases (87-89). Knowing if persons with a history of AN have increased central adiposity and could therefore be at increased risk for chronic diseases associated with TrFM could help medical practitioners provide more appropriate care for these individuals.

2.4 Cortisol

Cortisol is a stress hormone that is rapidly released, in response to physical or psychological stress, from the adrenal glands and is regulated by the hypothalamic-pituitary-adrenal (HPA) axis. Disturbances in the HPA axis are associated with alterations in cortisol levels (73,74,98). For example, Addison's disease is characterized by a lack of cortisol and with weight loss (98), while Cushing's Syndrome is associated with high cortisol levels and increased obesity (73,74).

Cortisol, which increases lipoprotein lipase (LPL) activity (99) leads to the accumulation of lipids, increasing adiposity in the form of visceral adipose tissue (VAT) and a relatively low amount of fat breakdown for utilization once the fat is stored (100,101). However, in starvation, cortisol mediates an increase of LPL which enables the use of more free fatty acids for energy (102), and cortisol increases protein catabolism (103). Furthermore, cortisol facilitates the release of energy, limits glucose use by inhibiting insulin action on insulin requiring tissues and is involved in gluconeogenesis and lipolysis (104-113).

Cortisol levels follow a circadian rhythm in which levels are highest in the morning, upon waking, followed by a mid-day increase and lowest levels in the evening (114-117). There are several factors that have been shown to effect cortisol levels in the morning and throughout the day (118). For example, the magnitude of morning cortisol increases differ between late and earlier risers (118). “Non-healthy” people have been shown to have a greater morning cortisol area under the curve than healthy people (118). In addition, it has been shown that alterations in sleep cycle and sleep deprivation are associated with increased serum cortisol levels (119-121). For example, one study shifted subjects’ sleep and wake cycle and found that the rising phase of cortisol was increased by 3 hours, decreasing the time of the trough in cortisol levels (119). Therefore, the normal cortisol cycle was altered by sleep changes (119). Circadian rhythm has been shown to be altered in some, but not all psychological disorders (122-124). For example, people with severe depression not only have higher cortisol levels, but the circadian rhythm is altered in terms of cortisol release (122). However in other psychological disorders, like AN, circadian rhythm may remain intact despite increased cortisol levels (123,124).

Patterns of cortisol secretion change throughout the day (117,125,126) with cortisol response to some stressors being greater in the afternoon and evening than morning hours (127-130). For example, adrenocorticotropic hormone (ACTH) stimulation tests have yielded greater cortisol responses in the afternoon and evening than the morning (127,128). Similarly, insulin produced a greater cortisol response in the afternoon or evening compared to the morning

(129,130). Cortisol levels rise following a meal (77,131-134) and therefore a "lunch stress test" (75,133-135) is often used to measure cortisol response to stress. In addition, the stress of a meal has also been shown to be greater in the afternoon than evening (136,137).

2.4.1 Cortisol, Central Adiposity, and Chronic Disease

Cortisol is associated with increased abdominal obesity (73-75,138) and increased risk for chronic diseases (74,75,120). For example, one study showed a correlation between sleep deprivation, increased cortisol levels and increased risk for type 2 diabetes (120). In other clinical studies, higher morning cortisol levels were associated with higher waist-to-hip ratio and lower HDL levels in men (76) and with higher insulin, glucose, and triglyceride levels (77). Rosmond and Bjorntorp showed a correlation between abnormal salivary morning cortisol variability, insulin, glucose, triglycerides, blood pressure, and abdominal obesity, all factors associated with the metabolic syndrome (75).

Increased cortisol levels are associated with increased central adiposity resulting from stress in women (138-140) . Women who secrete more cortisol during a corticotrophin-releasing factor / arginine-vasopressin test have increased VAT, compared to women who secrete lower amounts of cortisol (138). Further, in women who expressed feeling stress resulting from a mental stress test, cortisol secretion was higher in women with higher VAT (138). Other studies have also shown that higher levels of cortisol resulting from mental stress are associated with increased central adiposity (139). Binge Eating Disorder has also been linked to higher levels of cortisol; within one study, higher cortisol

levels under stress were associated with increased abdominal fat for people with binge eating disorder (140).

2.4.2 Cortisol and Starvation

Adults who were born SGA are reported to have higher cortisol levels both in the baseline and stressed states compared to those born adequate for gestational age (AGA) (8,141-148). However, results from other studies suggest there is no difference in cortisol levels between those born SGA and AGA peers (149-151). Still, results from other studies have shown lower cortisol levels in persons born SGA (152,153). Stunted children have been shown to have higher stress cortisol levels (154). However, another study showed stunted children to have similar baseline cortisol levels with a blunted stress response compared to non-stunted peers (155). Despite inconsistent data on persons born SGA and stunted children, it seems that alterations in cortisol may occur following undernutrition in early childhood. However, the effects of undernutrition on the HPA axis could be different than those who experience undernutrition in adolescence or adulthood. Further studies are needed to elucidate the relationship between cortisol levels and undernutrition in adolescence or adulthood.

In adults, caloric restriction and cortisol levels have been studied in several different groups of women (2,4,124,156-163). Fasting results in increased serum cortisol levels in normal weight women (156,157). However, caloric restriction has no effect on serum cortisol levels in obese women (158,159). One study of women reported a normal cortisol response to a

dexamethasone suppression test before and following weight regain and stabilization, but not during a three week fast (164). While weight prior to caloric restriction may have an influence on cortisol levels during or following caloric restriction, further studies are needed to determine the long-term impact of severe and sustained weight loss, like that seen in AN, followed by weight recovery on cortisol levels.

2.4.3 Cortisol levels and Eating Disorders

Several groups have studied cortisol levels in persons at entry into treatment for AN and following short-term weight regain (2,4,123,124,160,161,163,165). Using serum, salivary, and urinary free cortisol, it has been shown that cortisol levels in persons with AN are higher than the control participants (2,4,123,124,160,161,163,165). dos Santos et al. found salivary cortisol to be higher in women with AN than control women at 9am, 5pm and 11pm (124). Kling et al. used a competitive glucocorticoid antagonist to induce a cortisol response in AN and control women; women with AN had an increased cortisol response in the afternoon compared to control women (165). In the re-feeding process, cortisol levels increased in AN subjects compared to control participants in response to a meal and was positively associated with the amount of calories fed, despite the participant being unaware of the amount of calories in the solution (166).

While little information is available about cortisol levels during re-feeding, data are inconsistent regarding cortisol levels in short-term weight regain from AN (2,4,124,160,161,167,168). It has been shown that 7 months after admission

to an ED unit with some weight recovery, cortisol levels decreased (167). Kling et al. showed that urinary free cortisol decreased from acute AN to short-term recovery and cortisol levels no longer differed from control women (165). Participants with weight restoration averaging slightly over 2 years, (though some participants were only in recovery for 6 months) showed plasma and cerebral spinal fluid cortisol levels that did not differ from control participants (168). However, restrained eaters in general, have been shown to have higher cortisol levels than unrestrained eaters (169). It has also been shown using urinary free cortisol and serum cortisol that cortisol levels remained higher in persons during re-feeding or short-term recovery following AN than persons without a history of an ED (2,4). Grinspoon et al. reported that cortisol levels were higher in persons with AN and cortisol and the ratio of percent TrFM to percent extremity fat were increased in weight regain from AN compared control subjects (2). Furthermore, higher cortisol was positively associated with increased TrFM gain during weight regain (2). In a recent study, Mayer et al. showed that cortisol levels were still higher in women in short-term recovery from AN women and these women had higher TrFM and VAT compared to control women (4).

Grinspoon et al. and Mayer et al. both examined cortisol levels in weight re-gain following AN, but neither study examined cortisol or cortisol stress response in long-term recovery from AN. In recovery from AN, cortisol levels following a meal do not rise as cortisol levels would normally rise following a meal (170). Studies have not examined the effect of mental stress on cortisol and cortisol stress response in long-term recovery from AN. Thus, alterations in

cortisol secretion may occur following AN. Greater cortisol levels, greater central adiposity, and an altered cortisol stress response have been shown to exist in persons in short-term weight recovery from AN compared to control women with no history of an ED (2,4,170). It is important to see if differences in baseline cortisol levels persist with long-term weight recovery as this group could be at increased risk for gain of additional central fat mass. Further, it is important to further elucidate the relationship between recovery from AN and cortisol stress response as this could also place this group at risk for further increased gain of central fat mass.

2.5 Resting Energy Expenditure

Resting energy expenditure (REE) is influenced by body composition and is proportional to the amount of LBM (171-173). Thus any loss in LBM noted in caloric restriction would result in a reduction in energy expended. As weight is regained, if FM is gained more quickly and to a greater degree than LBM, lesser increases in REE would occur. As a lower REE has been associated with gain of FM (174), if long-term recovery from AN is associated with low REE, recovering women could be at increased risk for gain of FM.

2.5.1 Resting Energy Expenditure in Previously Undernourished Populations

REE has been studied in both persons born SGA and stunted children (9,10,175-178). For example, persons born SGA have lower a REE compared to those born AGA, with a trend for lower REE adjusted for LBM (10). However, it has also been reported that persons born SGA have higher REE than persons born AGA (175), even when adjusting for LBM (175). Similar to persons born

SGA, data from studies on stunted children have also varied in that some have showed stunted children to have lower REE than non-stunted peers when REE was not adjusted for LBM (176,177). In one study it was noted that the lower REE could be due to the lower LBM in the stunted group (177). It has also been shown that there is no difference in REE adjusted for LBM between stunted and non-stunted children (9,178). Overall, when adjusting for LBM, there do not seem to be difference in REE between people who experienced undernutrition in utero or in early childhood compared to those who did not.

2.5.2 Resting Energy Expenditure and Anorexia Nervosa

In AN REE is lower than in women without a history of an ED (179-184). However, most of these studies failed to properly control for LBM (179-182,184). “Near death” persons with AN have REE values higher than expected, but this group is different than the majority of persons suffering from AN due to the extreme low weight (185). The difference in REE could be due to the body’s preservation of LBM on which REE is greatly dependent. Thus, overall persons with AN seem to have relatively low REE values compared to healthy individuals, but further studies are needed to determine if differences persist when adjusting for LBM.

Generally, there is a consensus that REE increases from acute AN to the re-feeding period (93,180,186-191). Although it has also been shown that post-re-feeding REE and REE adjusted for lean body mass remain lower than women without a history of an ED (183). In extreme starvation REE has been shown to be higher than expected and then decrease with re-feeding (185).

Timing of REE measurement is likely important as studies have shown initial increases in REE in early re-feeding that stabilizes over time (188). Further, REE increases as LBM increases, an observation that could occur over the entire period of weight regain, but the highest increases have been noted earliest in the weight regain process (192). Therefore the time-point at which REE is measured in the weight regain process can greatly influence the results. In general, if correcting for LBM, these differences may no longer exist. Further studies are needed to determine if REE is similar in recovering and control women while adequately controlling for body composition.

2.6 Low Fat Oxidation is Associated with Increased Fat Mass

Low fat oxidation is associated with increased central fat storage as less fat metabolized from the diet promotes fat storage (193,194). The Baltimore Longitudinal Study for Aging found a higher respiratory quotient (RQ), indicating lower fat oxidation and higher carbohydrate utilization, was associated with increased body fat mass (195). It has also been noted that Pima Indians a with low rate of fat oxidation (higher RQ) also have a higher percent FM than those with a lower RQ (194). Stunted children with a higher fasting RQ, compared to non-stunted peers (9), gained more FM over a three year period (196). In addition, results from one study have shown a trend for lower fat oxidation (10) and higher central adiposity in persons born SGA compared to peers born AGA (5,6). Therefore, impaired substrate oxidation could be a mechanism leading to increased central adiposity in groups that experience undernutrition.

2.6.1 Substrate Oxidation and Weight Change

While in the early 1900's Benedict et al. showed that RQ did not differ during caloric restriction in young men (197), using a single subject it has been shown that RQ decreased in the early days of the starvation and remained below 0.80 for the duration of the starvation (198). More recently it has been shown that while malnourished adults have increased fat oxidation (184,199), fat oxidation decreases with weight gain (184,200). However, in one study RQ remained lower, though not statistically significantly lower, than that of control volunteers (RQ = 0.84 v. 0.90, respectively) (184). Therefore, although there was a change in substrate oxidation from when participants were sick and underweight to when they were in a healthy state, in a healthy state substrate oxidation did not differ from a control sample (184). Overweight or obese adults who have lost and regained weight have low fat oxidation following weight regain (201). Data from other studies show that obese persons who lose weight have higher RQ values than lean individuals of a similar BMI (202,203). Hence, it appears that baseline body composition and total body weight may also be factors that influence if and how weight change will result in altered substrate oxidation.

2.6.2 Anorexia Nervosa and Substrate Oxidation

Substrate oxidation may or may not be different in women with AN compared to control women (181,184,188,204). In a fasted state during AN, plasma glucose levels are lower than control participants (205,206) and free fatty acids do not differ between groups (206). It has been shown that RQ does differ

between women with AN and control women (181,184,204). However, it has also been shown that RQ is higher in women with AN (188).

Differences in RQ between AN and control participants may be due to differences in actual fuel utilization. For example, carbohydrate oxidation has been shown to be comparable (204,205) or higher (188,206) in AN compared to control participants. Similarly, protein oxidation has been reported to be comparable (184,188,206) or higher in AN participants (205). Finally, fat utilization has been shown to be comparable (204) or lower (188,205,206) in AN than control participants. Thus far, studies have not controlled for diet (188,204-206) and dietary differences could lead to differences in fuel utilization noted between studies. Therefore, further studies are needed in order to determine the relationship between AN and fuel utilization while controlling for diet.

In the re-feeding process, changes in fuel utilization may occur. Since macronutrient intake differs in person with AN compared to control participants (181,207,208), it is not known if re-feeding is associated with a shift in macronutrient composition of the diet along with increased caloric intake, which could alter RQ values. Substrate oxidation has only been studied during re-feeding or shortly following re-feeding from AN (180,181,183,184,188,204-206). In re-feeding non-protein RQ (NPRQ) exceeds that of AN and control participants (204). Carbohydrate, and not fat, utilization is increased during in the initial weeks of re-feeding period in AN (204).

Following re-feeding, changes in substrate oxidation may again occur. Immediately following re-feeding, AN participants did not have RQ values that

differed from control participants (183,184). Protein oxidation was also similar between groups shortly following re-feeding (184). Approximately 6 months following initial measurement, after re-feeding, percent of REE contributed to by fat utilization has been shown to be increased and that of carbohydrate utilization decreased compared to fat and carbohydrate utilization at admission (188).

RQ data during AN, in re-feeding from AN, and in short-term weight regain following AN are inconsistent (181,184,188,204,205) and data regarding RQ in long-term weight regain following AN has not been thoroughly studied. It is possible that, in long-term weight recovery in a RAN population, impairments in fat oxidation occur similar to stunted children. Impaired fat oxidation is a possible mechanism for increased body fat accumulation (193,194). However, short-term weight stabilization has been associated with increased, not decreased, fat utilization in persons with a history of AN (188). Therefore, the aim of this study was to determine if increased fat utilization seen shortly after weight recovery persists and is different than that of control participants or if long-term recovering subjects oxidized fuel similar to stunted children, thereby placing them at increased risk for central fat gain.

2.7 Perfectionism and Anorexia Nervosa

While the stress of starvation could contribute to both short and long-term increases in cortisol levels in persons with a history of AN, psychological factors could also contribute to increased cortisol levels in this population. Perfectionism is common in persons with AN (209-213). A recent study has found an association among three genes, perfectionism, and AN (214). Not only is

perfectionism higher in AN (209-213), but it seems to have important treatment implications (215,216). In a longitudinal study it has been shown that persons with AN that scored higher on perfectionism took longer to enter into remission from AN (215). Another study that used the Eating Disorder Inventory perfectionism scale found that persons with higher perfectionism did less well in treatment than persons with lower perfectionism (216).

There are different components of perfectionism that have been measured in AN (213,217-220). Persons with AN score higher than persons without EDs on self-oriented, but not socially-oriented perfectionism (213,217-219). In one study almost 20% of persons with EDs scored 2 standard deviations above the mean for control participants (217). Using the Perfectionistic Self-Presentation Scale (PSPS), it has been shown that persons with AN not only score higher on overall perfectionism, but also each of the 3 individual components, perfectionstic self-promotion, nondisplay of imperfection, and nondisclosure of imperfection, of perfectionism assessed by this scale (220).

Studies have shown that perfectionism tends to persist in both short and long-term recovery from AN (65,170,213,215,216,221). For example, 8 and 16 years after AN diagnosis, perfectionism levels remained constant despite recovery from AN (215). After over 1 year of recovery from AN, persons with a history of AN scored higher than control women on two different measures of perfectionism (221). Sullivan et al. showed that 12 years after on-set of AN, those in recovery from AN still scored significantly higher on the Eating Disorder

Inventory perfectionism scale compared to control participants (65). Thus perfectionism may not dissipate despite recovery from AN.

2.7.1 Perfectionism and Cortisol

Thus far few studies have examined perfectionism and cortisol (170,222,223), and few examined perfectionism, cortisol and AN (170,223). Using the Trier Social Stress test (TSST) to induce stress, a recent study has shown that in men, higher perfectionism is associated with higher cortisol response to stress (222). One study has shown that women with a history of AN still score high on perfectionism, but lack the increase in cortisol that normally accompanies a meal (170). Another study, which included both measures of cortisol and perfectionism, but lacked a control group, examined how cortisol and perfectionism (independent of one another) changed over treatment in persons with AN compared to persons with bulimia nervosa (223). In persons with AN neither perfectionism nor cortisol levels decreased significantly over the course of the study (223). Thus it is likely that continued perfectionism in RAN individuals could contribute to increased cortisol levels at baseline and in response to stress. The increased cortisol levels could thereby contribute to increased risk for central adiposity tissue gain.

2.8 Body Satisfaction

Many persons with AN experience body dissatisfaction (224-228). Extreme weight and shape concerns are part of the core psychopathology and diagnostic criteria for AN (20,229,230). One study compared weight and shape concerns in women with AN to restrained and unrestrained eaters and found that

AN subjects had greater body dissatisfaction than both comparison groups (231). Both therapists and persons with eating disorders agree that addressing body image concerns are important for treatment of EDs (232). Higher body dissatisfaction has been shown to be positively associated with relapse in both AN and bulimia nervosa (233). However, following first diagnosis of AN, body image distortion has also been shown to be the same in women who successfully recover from AN as those who relapse (234). Further, a woman with a history of AN who overestimates the size of the her hips is more prone to relapse (234). Still, body dissatisfaction or poor body image can persist into recovery from AN (235).

The Body Shape Questionnaire-34 (BSQ) has been shown to be a valid tool for measuring body satisfaction (236-238). Even in a non-eating disorder sample, higher weight is associated with an increased score on the BSQ indicating higher body dissatisfaction (239). Despite the BSQ being a valid tool to study body image, at this time, it has not been used to study the relationship between body satisfaction and cortisol levels. If body dissatisfaction and poor body image persist into recovery, it is possible that this psychological stress could be associated with increased cortisol levels.

Using other measures of body satisfaction, it has been shown that persons with EDs show higher negative emotion surrounding body image (240). It has also been shown that persons who are more concerned about their body image have higher afternoon cortisol levels (241). A negative correlation has also been found between body esteem and waking cortisol levels (242). It is

possible that the stress of body dissatisfaction could increase cortisol levels in women recovering from AN. While body dissatisfaction could be associated with increased cortisol levels (241,242), this has not been studied in a population of women in long-term recovery from AN. If poor body image is correlated with increased cortisol levels, this could further demonstrate body satisfaction to be an important area to target in the treatment of AN.

3 RATIONALE

3.1 Significance of Research

According to the Academy for Eating Disorders, approximately 0.5-1% of the female population suffers from anorexia nervosa (AN) (1). While it is known that women in short-term recovery from AN have higher cortisol levels and higher central adiposity than women without a history of an eating disorder (4), little is known about the long-term effects of AN. It is possible that the time following undernutrition in adolescents and adults could influence body composition, body fat distribution, cortisol levels, cortisol response to stress and energy metabolism. Determining if women in long-term recovery from AN have alterations in body composition, altered body fat distribution, increased cortisol levels in a baseline state and in a stressed state, altered cortisol response to stress and altered energy metabolism could lead to improved medical and psychological care for people who experience AN.

3.2 Importance of Research

The results of the present study will help in the understanding of the relationships between body composition, body fat distribution, cortisol levels in both baseline and stressed states, cortisol response to stress, resting energy expenditure (REE) and recovery long-term from AN. Perfectionism and body satisfaction will be assessed as these are two psychological factors that could increase cortisol levels and be potentially important targets in the treatment of AN. High cortisol levels, impaired fat oxidation and low REE could be associated with increased body fat and central adiposity (138-140,174,193,194). Hence it is

important to study the relationship between these factors in individuals who have experienced undernutrition following early childhood. Results from this study are likely applicable to other groups that experienced some type of starvation following early childhood, such as famine victims, cancer survivors with weight loss from chemotherapy, as well as persons who suffered from other diseases associated with extreme weight loss and recovery.

4 HYPOTHESES AND SPECIFIC AIMS OF THE RESEARCH

4.1 Hypotheses

The central hypothesis of this proposal is that persons in recovery from anorexia nervosa for 2 or more years (RAN) will be similar to persons born small for gestational age, stunted children and persons in short term recovery from anorexia nervosa (AN) and will have several risk factors for gain of central fat mass and thereby risk factors for chronic diseases. We hypothesize the following:

1. Persons with a history of AN will have similar fat mass, but increased truncal fat mass compared to persons with no history of an eating disorder (ED).
2. Persons with a history of AN will have higher cortisol levels in a baseline, morning, state compared to persons with no history of an ED.
3. Persons with a history of AN will have higher cortisol levels in a psychological stressed state and a higher cortisol response to stress than persons with no history of an ED.
4. There will be no differences in resting energy expenditure between persons with a history of AN and control participants.
5. Persons with a history of AN will have lower fat oxidation, indicated by higher respiratory quotient values than healthy peers with no history of an ED.

In addition, we hypothesize that the increased cortisol levels could be due in part to RAN participants having higher perfectionism and higher body

dissatisfaction than persons with no history of an ED. These personality characteristics could potentially raise cortisol levels at baseline or in a stressed state. Therefore we will evaluate body satisfaction and perfectionism in RAN and control participants.

4.2 Specific Aims

The aim of this study was to determine if long-term recovery from AN is associated with changes in body composition, body fat distribution, cortisol levels, cortisol response to stress, and energy metabolism. In addition, we examined perfectionism and body satisfaction as these are two factors that could be associated with increased stress and thus increased cortisol levels. All aims were studied by comparing RAN women to a control group of women with no history of an ED.

5 METHODOLOGY AND EXPERIMENTAL DESIGN

5.1 Subjects

Subjects were 16 women with a history of anorexia nervosa (AN) who were in recovery for 2 or more years (RAN) and 18 healthy control (C) women without a history of eating disorders (ED). All subjects were between 18 and 35 years of age with a body mass index (BMI) ≥18.5, were weight stable ± 2.2 kg for 3 months, non-smokers, free from chronic diseases (e.g., Diabetes, Cushing's disease, or Crohn's disease) and not taking any medications that are known to influence metabolism or body composition (including but not limited to diet pills, steroids, or beta blockers). Subjects were recruited via flyer advertisement, email advertisements and in-class announcements at Rutgers University. To assess each subject's history of AN, a screening questionnaire consisting of psychological variables indicative of AN, lowest lifetime weight, and menstrual history was administered. Such questionnaires have been found to be valid and have been used in numerous studies of eating disorders (69,170,243-253). All subjects met the *Diagnostic and Statistical Manual of Mental Disorders, IV edition* (DSM-IV) criteria for AN two or more years prior to initial screening (20) except for three subjects. Those three subjects received a diagnosis of AN following loss of greater than 15% of her body weight in a short time (e.g. 4-6 weeks) without having BMI fall below 17.5, consistent with the International Statistical Classification of Diseases and Related Health Problems 10 (ICD-10) diagnosis of atypical AN (254). The precise age at which AN occurred was not readily available for all women. However, each RAN participant was post-

menarchal and at her adult height when she experienced AN. Recovery was defined as having a BMI ≥ 18.5, being weight stable for 3 months or greater, absence of binging or purging, absence of another eating disorder, and exercise not exceeding that recommended by the United States Department of Agriculture 2005 Dietary Guidelines (255). Except for one RAN and one C subject, all subjects had regular menses. The RAN subject was unable to menstruate without the use of oral contraceptive drugs and chose not to take the drugs and the C subject continually took oral contraceptives without taking the placebo to avoid menstruation.

The protocol used in this study was approved by the Rutgers University Institutional Review Board for the Protection of Human Subjects and informed written consent was obtained from all subjects.

5.2 General Protocol

The study design is outlined in **Table 5-1**. At the time of screening, questionnaires to assess history of eating disorders, recovery from eating disorders and general health and the Body Shape Questionnaire- 34 (BSQ) were completed by each participant. Height was measured to the nearest 0.1 cm using a stadiometer (Invicta Plastics Ltd., Oadby, UK) and weight was measured to the nearest 0.1 kg on a digital scale (Tanita BF 578, Tokyo, Japan). At the time of measurement, each subject was wearing light clothing and socks. Height and weight measurements were taken at the screening and two days prior to the study day.

Following the initial screening subjects were scheduled to begin the research protocol. To ensure consistency with cortisol levels, all volunteers were measured during the luteal phase of the menstrual cycle (256-259), which has been shown to have the highest cortisol levels and has been utilized by other studies (156,259-261). Actual study participation began with subjects signing an informed consent and each subject was given the instruments to perform the at-home saliva collection, instructions regarding meal consumption the day before the study, and tickets for the meals which would be picked up and consumed the day prior to the study. To establish baseline values, blood pressure and heart rate were taken three times, 3 minutes apart in the non-dominant arm using Omron HEM 705 CPN Auto Inflate Blood Pressure Cuff (Bannockburn, IL).

5.3 Body composition

Body composition was assessed using dual energy x-ray absorptiometry (DXA) (Lunar Prodigy Advanced DXA GE-Lunar, Madison, WI) with enCORE 2004 software (version 8.10.027). Each subject was positioned on the DXA using a standardized protocol for positioning of the head, torso and limbs, was told to remain still while a whole body scan, taking less than 15 minutes, was performed. The DXA software automatically divided the body into total body, extremities, trunk (shoulders out to ribs and down to femoral neck) (262-265), android, and gynoid regions. Total tissue fat was used for each region.

5.4 Cortisol Collection Protocol

Salivary cortisol has been shown to be a valid method by which to measure baseline and stressed state cortisol levels and was used here because

it is been utilized in other studies, is safe, relatively easy and effective (8,266-270). In order to obtain salivary cortisol samples, each subject was instructed to chew on the cotton piece until the cotton was thoroughly saturated and place it in the salivette provided. All samples were collected on a normal weekday when the subject was not under any unusual stress and each subject was told not to consume alcohol for 12 hours prior to taking the first sample.

5.4.1 Baseline Cortisol Collection Protocol

Baseline salivary cortisol samples were collected at home by the subjects and this allowed us to obtain cortisol levels from subjects at various times of an unremarkable day without intrusion from the researchers which might have increased cortisol levels. Subjects were asked to collect saliva at home the day prior to the study upon awakening, 15 minutes later, 30 minutes later, at 3pm and at bed time. Subjects were given five salivettes with a 1 inch piece of cotton rope in each salivette and instructed to chew on the cotton until the cotton was thoroughly saturated and return it to the salivette. Samples were returned to the researcher and frozen at -20^{o}C until analyzed (8,266).

5.4.2 Trier Social Stress Test

In women, mental stress has been shown to produce a salivary or urinary free cortisol stress response (139,271-273). While many studies have utilized a “lunch” stress (75,133-135) this had higher variation in response to time of day than the TSST (136,137,274). Further a relationship between salivary or urinary free cortisol stress response to psychological stress and central adiposity has been shown in women (139,271,273). Therefore in this study, subjects

performed the Trier Social Stress Test (TSST) which has been shown to produce a cortisol stress response in different populations and at different times of the day (261,274-279).

Subjects were instructed to go about normal activities prior to the stress test measurement, but told to fast for 90 minutes before arriving for the measurement. The TSST has been shown to produce a cortisol stress response in the morning and afternoon (274). Since cortisol follows a circadian rhythm (280), each TSST was conducted between 1 and 5pm. Time at which the TSST was performed can be controlled for statistically (274,281).

Upon arrival, each subject rested for 15 minutes prior to performing the TSST, which has been shown to produce a cortisol stress response (274-276,279). A saliva sample was obtained as earlier described and the subject was asked to rate on a scale of 1-10 how stressed she was feeling at that time. After obtaining the initial saliva sample, a Polar FS3 heart rate monitor and a blood pressure cuff was fitted on each subject for the duration of the measurement according to manufacturers' instructions. All blood pressure measurements were taken in the non-dominant arm. Following the initial heart rate and blood pressure measurement a second saliva sample was obtained and the subject was again asked to rate how stressed she was feeling.

To perform the TSST, the subject was told she was to imagine she was at a job interview that was being audio and video recorded. In addition, 2 researchers unknown to the subject were present. Each subject was given 3 minutes to prepare a speech on why they deserved the position for which they

were applying for and were told to remain standing while taking notes. Each subject was told that the notes could not be used during the speech and that during the speech researchers may interject if they disagree with the subject or wanted more information. The subject was also told another test would follow the speech.

After 3 minutes the notes were taken from the subject and the subject had 5 minutes to give a speech for the job interview. The subjects had to speak for the full 5 minutes. Questions were asked by the researchers to ensure that each subject remained talking for the full five minutes. Each subject was interrupted by researchers at least one time.

In the second task, the subjects had 5 minutes to count backwards from 2023 in increments of 17. The subjects were told that the researchers would tell them if a mistake was made and they would be asked to start over. Each mathematical test was conducted for a 5 minute period of time.

Saliva samples were collected as earlier described immediately following the TSST, and 10, 20, 30, and 45 minutes post TSST (8,259,276,279,281-284). At each cortisol collection time point, heart rate, blood pressure, and stress level (subjective on a scale of 1-10) were recorded. Ten minutes following the completion of the oral portion of the TSST participants were debriefed and told that her interview had not actually been recorded. In addition, 13 minutes following the TSST, each subject stood for 12 minutes to measure recovery heart rate (8,259,276,279,282-284).

5.4.3 Cortisol Analyses

Cortisol samples were analyzed using the Expanded Range High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit (Salimetrics, LLC, State College, PA). The assay was performed according to manufacturer's instructions for each sample (266,285-288). Saliva samples were centrifuged at 3000 rpm for 15 minutes. 25 µL of 8 control solutions, assay dilluent and each sample were pipetted into the appropriate wells and incubated for 1 hour with 200 µL of conjugate solution pipetted 50 µL at a time using a multi-channel pipette. Samples were disposed of and the plate was washed 4 times using a 1X buffer solution. 200 µL of tetramethylbenzidine solution was added 50 µL at a time using a multi-channel pipette. The plate was covered with aluminum foil and incubated for 30 minutes. 50 µL of stop solution was added using a multichannel pipette and the plate was incubated for 3 minutes after which time the plate was read in a plate reader with wavelength set to 450 nm. Calculations to obtain cortisol values were made using the following equation provided by Salimetrics, LLC: 10^(y = mx + b); y is percent bound (B/BO), b is the intercept, and m is the slope, x (Log of Conc.) = (y - b)/m.

5.5 Energy Metabolism Protocol

Resting Energy Expenditure (REE) and Respiratory Quotient (RQ) were measured for each subject. To ensure that measurements were not influenced by their diet the day prior to the study, all subjects were provided with all meals and snacks the day before measurements were taken and instructed not to engage in vigorous physical activity. Meals were provided by a dining hall at the

university. Macronutrient composition of the non-vegetarian diet was approximately 50% carbohydrate, 32% fat and 18% protein and the vegetarian diet 52% carbohydrate, 35% fat and 13% protein. Caloric requirements were estimated with using the Mifflin equation: REE = -161 + (10*weight (kg)) + (6.25*height (cm)) – (5*age (yrs)) with an activity factor of 1.4 (low to moderately active) (289-291). In AN and re-feeding the Mifflin Equation's prediction was within 10% of basal metabolic rate measured (292).

Two days before measurements were conducted, subjects were given a list of food items to be provided by the dining hall and instructed to consume each item on the list and to not consume additional food or beverage items. Each subject was further instructed to cross off each item as each item was consumed and return the list to the researcher. Actual caloric consumption was calculated using the nutritional information provided by the dining hall and each food quotient (FQ) was calculated using the following equation: FQ = (0.207*%carbohydrate + 0.159*%fat + 0.193*%protein) / (0.207*%carbohydrate + 0.226*% protein + 0.243*%fat) (293).

5.5.1 Indirect Calorimetry

REE and RQ were measured using indirect calorimetry (IC) (VMax Spectra 29N, Sensormedics, Inc., Yorba Linda, CA). Subjects arrived at the university in a fasted state, were scheduled to arrive shortly after the time the subject usually arises and instructed not to engage in any physical activity prior to the measurement. Upon arrival, each subject rested in a bed for 30 minutes while the IC was calibrated. A transparent hood was placed over the subject's

head and IC was conducted for 30 minutes while the subject lay quietly avoiding all types of motion. The last 20 minutes of each measurement were used to estimate the REE and RQ. The IC was calibrated before each measurement using a standard mix of carbon dioxide (4%) and oxygen (16%) and REE was estimated as follows: REE (kcal) = 3.781*VO_2L+ 1.237 *VCO_2L (294). RQ value was determined by IC using the following equation: VCO_2/VO_2.

5.6 Body Satisfaction and Perfectionistic Self-Presentation Scale Questionnaires

Subjects completed the Perfectionistic Self-Presentation Scale (PSPS), a tool designed to measure perfectionism and the BSQ, a questionnaire designed to measure body satisfaction. Both the PSPS and the BSQ tools have been shown to be valid, yield internally consistent results, are consistent with other similar measures and have been used previously in eating disorder populations (219,236-238,295). The PSPS has been demonstrated to have reliability and test re-test reliability (219). Overall perfectionism and the three subscales of the PSPS: perfectionstic self-promotion, nondisplay of imperfection, and nondisclosure of imperfection were also studied (213,217-219). The PSPS was completed at the time of the DXA scan.

BSQ was utilized as a whole, in addition, questions 15 and 30 were analyzed individually as these are indicative of body checking and body avoidance (296). Previous studies have found a score of 90 to be indicative of normative body dissatisfaction (237,239), therefore, we utilized this value for normative body dissatisfaction.

5.7 Statistical Analyses

Power analyses were completed using Power and Sample Size Calculations Version 2.1.31 (297) and are contained in **Table 5-2a-b**. The following statistical analyses were performed to help accomplish each of our specific aims.

Using a skewness test, data were determined to be normally distributed when the skewness calculated was less than twice its standard error. All data except cortisol and BSQ score were normally distributed. Student's t-test was used to determine if between RAN and C subjects existed for baseline and all outcome variables.

To determine the relative strength of significant differences, effect size was calculated using Cohen's *d* and Pearson's *r* for student's t-tests or partial Eta squared for multiple linear regression equations. In accordance with previously established standards, an absolute value for Cohen's *d* of 0.50 was considered to have a medium effect and an absolute value for Cohen's *d* of ≥ 0.80 a large effect (298). All analyses were conducted using SPSS 14.0 for Windows (SPSS Inc, Chicago, IL) and a p-value ≤ 0.05 was considered statistically significant.

5.7.1 Body Composition

To determine if there was a relationship between fat mass (FM) and/or body fat distribution and recovery from AN, multiple linear regression analyses were conducted. Truncal Fat Mass adjusted for FM (AdjTrFM) was constructed from the residuals of truncal fat mass (TrFM) regressed on FM. The following regression analyses were conducted: 1a) FM = β + Lean body mass (LBM) +

group; 1b) FM = β + LBM + group + height; 1c) FM = β + LBM + group + height^2; 2) AdjTrFM = β + LBM + group + height^2; 3) TrFM:FM = β + LBM + group + height^2.

5.7.2 Cortisol

Cortisol levels were transformed to the natural log equivalent. The awake + 30 cortisol level was normally distributed and therefore this value was used in the regression analyses. One C subject was found to be an outlier falling more than two standard deviations from the mean for cortisol levels and removed from cortisol analyses.

The following multiple linear regression was performed to determine in the effect of exercise and group on heart rate at baseline, for each TSST time point, average heart rate and highest: heart rate = β + group + hours of exercise. There was no interaction between group and hours of exercise. Rate of change for heart rate was calculated as follows: (Δ heart rate B to A) / (Δ time B to A (minutes)). General linear model (GLM) repeated measures was performed to determine the relationship between change in heart and recovery from AN. Each of the six TSST time points were entered as within-subject variables and group was entered as the between subject variable. Pillai's Trace was used to determine if there was a significant change in heart rate and if there was a group effect. GLM repeated measured were utilized to determine if heart rate changed significantly between individual time points and if there was a group effect.

Rate of change for cortisol was calculated as follows: (Δ cortisol B to A) / (Δ time B to A (minutes)). GLM repeated measures were performed to determine

the relationship between change in cortisol and recovery from AN, here the natural log variable for each cortisol time point was used. Natural log of each of the seven TSST time points were entered as within-subject variables and group was entered as the between subject variable. Pillai's Trace was used to determine if there was a significant change in cortisol and if there was a group effect. GLM repeated measured were utilized to determine if cortisol changed significantly between time points and if there was a group effect.

Multiple linear regression analyses were used to determine if use of oral contraceptives had an influence on cortisol levels at baseline or during stress and if time of day at which the TSST was conducted had an influence on cortisol levels during stress. Dummy variables were created for each of the 3 times at which the TSST was conducted as follows: Dummy 1 = (1:30pm = 1 and 3pm and 4:30pm = 0); Dummy 2 = (3pm = 1 and 1:30pm and 4:30pm = 0) and Dummy 3 (4:30pm = 1 and 1:30pm and 3:30pm = 0). The following regression equation was performed for each cortisol sample obtained: 1) natural log cortisol = β + group + use of oral contraceptives and the following regression equation was performed for each cortisol sample obtained during the TSST 2) natural log cortisol = β + group + dummy 1 + dummy 2 + dummy 3. Neither use of oral contraceptives nor time at which TSST was conducted had a significant influence on cortisol levels so all TSST groups were analyzed together to allow for higher power to detect differences in outcome measures between groups.

To determine the relationship between factors known to influence cortisol levels, recovery from anorexia nervosa, and highest baseline cortisol level

(awake +30) the following regression analysis was performed: awake +30 = β + BSQ + PSPS + group. To determine the relationship between percent TrFM, recovery from AN and awake +30 cortisol levels the following regression equation was performed: percent TrFM = β + group + awake +30.

Area under the curve (AUC) was calculated in order to determine if there was a difference in cortisol response to the TSST. AUC was calculated using the SPSS syntax for trapezoidal factorial approach where $AUC_K = AUC_{Ki...n}$ + [time (TSST time point (1-7)) – lag time)*(lag cortisol + cortisol (TSST time point 1-7)] / 2 (222,274,299). AUC was determined to be skewed and transformed into its natural log for use in regression analyses. ANOVA was utilized to determine the relationship between cortisol stress response and recovery from AN.

Multiple linear regression analyses were performed to determine the relationship between time of day at which the TSST was conducted or the use of oral contraceptives and AUC: 1) natural log AUC = β + group + use of oral contraceptives and 2) natural log AUC = β + group + dummy 1 + dummy 2 + dummy 3. The use of oral contraceptives or the time at which TSST was conducted did not have a significant influence on cortisol levels so all groups were analyzed together to allow for higher power to detect differences in outcome measures between groups.

To explore the relationship between cortisol stress response and other factors known to be associated with increased cortisol levels and known to be higher in persons in recovery from AN, the following regression analysis was conducted: natural log AUC = β + group + BSQ + PSPS. To further explore the

relationship between recovery from AN, cortisol stress response and body composition the following regression equations were performed: percent TrFM = β + group + AUC. Partial eta squared was calculated for independent variables that were statistically significant for each regression equation.

5.7.3 Energy Metabolism

To determine if there was a relationship between energy metabolism and recovery from AN multiple linear regression analyses were utilized. The following regression analyses were conducted: 1) RQ = β + FQ + LBM + FM + group and 2) REE= β + LBM + FM + group. Effect size was calculated using partial Eta squared.

5.7.4 Questionnaires

Student's t-test was used to determine if differences in scores on the BSQ and questions 15 and 30 on the BSQ differed between groups. In addition, Student's t-test was used to determine if differences in scores on the PSPS or PSPS subscales differed between groups.

Table 5-1 Overall Study Design

	Study Day 1	Study Day 2	Study Day 3	Study Day 4
Screening	X			
BSQ	X			
Equipment Pick-up/ Explanation		X		
Blood Pressure Measurement			X	
RQ				X
TSST				X
Body Composition				X
PSPS				X

Table 5-2a Original Power Analysis

Variable	Expected Difference in Group Means	Standard Deviation	Estimated Sample Size per Group (80%/90%)
Truncal fat (%)	5.8	4.45	10/13
Cortisol	5.64	5	13/18
Resting Energy Expenditure	246	169	8/11
Respiratory Quotient	.04	.05	26/34

Table 5-2b Current Power Analysis

Variable	Expected Difference in Group Means	Standard Deviation	Participants per Group[1]	Power 1:1 ratio assumed	Power C:RAN[2]
Truncal fat (%)	5.8	4.45	16/18	96%	96%
Cortisol	5.64	5	14/11	71%	76%
Resting Energy Expenditure	246	169	16/17	98%	98%
Respiratory Quotient	0.04	0.05	16/17	59%	60%

[1]Women in recovery from anorexia nervosa for 2 or more years/control women
[2]C = Control; RAN = women in recovery from anorexia nervosa for 2 or more years

6 MAIN EXPERIMENTS

SECTION A BODY FAT DISTRIBUTION IN LONG-TERM RECOVERY FROM ANOREXIA NERVOSA

Abstract

Background: Anorexia nervosa (AN) is characterized by loss of fat mass. In short-term recovery from AN, women have similar total fat mass (FM), but higher truncal fat mass (TrFM) than control subjects. However, little is known about the long-term effect of AN on body composition and body fat distribution in long-term recovery from AN.

Objective: The aim of this study was to determine if increased TrFM reported in short-term recovery from AN exists in long-term recovery from AN.

Design: Dual energy x-ray absorptiometry was used to measure body composition in 16 women recovering from AN for 2 or more years (RAN) and 18 Control (C) women.

Results: RAN and C women had comparable weight (61.0 ± 8.1 kg and 63.5 ± 8.6 kg) and percent body fat (29.8 ± 7.2% and 32.1 ± 6.4%, respectively). No group differences were found for TrFM (27.2 ± 10.1% and 30.5 ± 8.0%, respectively) or TrFM adjusted for FM ($p = 0.655$).

Conclusion: In long-term recovery following AN, FM and TrFM do not differ from that of women without a history of an eating disorder.

Introduction

Anorexia Nervosa (AN) affects approximately 1% of the female population in Westernized societies (1,21-23) and is characterized by a refusal to maintain a body weight in a healthy range (20), often low percent body fat (4,72,93,94,96,185,300), an intense fear of gaining weight, and denial of the seriousness of the current low body weight (20). The low body weight characteristic of AN creates serious health risks, making it among the most dangerous psychological disorders (14,15). Gain of weight and fat mass (FM) are essential for recovery from AN, but also pose important challenges to treatment (97,301). While several studies have examined body composition and body fat distribution following initial weight gain in persons with AN (2-4,90-92) few have studied body composition in long-term recovery following AN, the aim of this paper.

Initial weight restoration in persons suffering from AN tends to result in a higher gain of FM compared to lean body mass (LBM), but final percent FM does not exceed than that of healthy, age-matched, control subjects (2-4,90-92). For example, 9 months following initial diagnosis, AN patients gained 68% of weight as FM, but their percent FM was still lower than control subjects (2). Here it must be noted that the AN participants were only at 81% of their ideal body weight, a body weight still classifying participants as having AN (2). In another study, 10 weeks following initial assessment, weight and FM of recovering AN subjects were both comparable to control subjects (4). Despite having a FM comparable or below that of control women, women with AN report feeling that fat is gained

disproportionally in the central region (97), an observation that seems to create additional treatment challenges (4,97).

Body fat distribution can have important health and treatment implications for recovery from AN. Thus far body fat distribution has only been studied in short-term recovery following AN. It has been reported that following initial weight restoration in persons with AN, percent truncal fat mass (TrFM) is higher than control subjects (3,4,71). Increased percent TrFM compared to control subjects has even been reported in AN subjects who have only gained a small amount of weight and still meet diagnostic criteria for AN (2). In general, these studies have varied in the criteria used to determine weight regain and often did not include long-term weight stabilization prior to final measurements (2-4,71). Therefore, it is possible that initial body composition and body fat distribution may not accurately represented long-term body composition and body fat distribution. However, little information is available on long-term body fat distribution in recovery from AN (96).

Determining if TrFM is higher in women in long-term recovery from AN compared to women without a history of an eating disorder can have important health and treatment implications. For example, if increased TrFM persists women with a history of AN could be at increased risk for cardiovascular disease or Type 2 diabetes (87-89). Furthermore, data on long-term body composition following AN could help clinicians better address clients' concerns about the gain of central fat mass. Therefore, the aim of this study was to determine if there are differences in body composition and body fat distribution in women in long-term

recovery from AN compared to healthy women with no history of an eating disorder.

Subjects and Methods

Subjects

Sixteen women with a history of AN and in recovery for 2 or more years (RAN) and 18 healthy control (C) women without a history of eating disorders were included in this study. All subjects were between 18 and 35 years old, had a body mass index (BMI) ≥ 18.5, were weight stable ± 2.2 kg for 3 months, non-smokers, free from chronic diseases (e.g. Diabetes, Cushing's disease, or Crohn's disease) and not taking any medications that are known to influence metabolism or body composition (e.g. diet pills, steroids, or beta blockers). All subjects were recruited via flyer advertisements, email advertisements and in-class announcements at Rutgers University. To assess each subject's history of AN, a screening questionnaire consisting of psychological variables indicative of AN, lowest lifetime weight, and menstrual history was administered (69,170,243-253). All subjects met the *Diagnostic and Statistical Manual of Mental Disorders, IV edition* criteria (DSM-IV) for AN 2 or more years prior to initial screening (20) except for 3 subjects who received a diagnosis of AN following losing greater than 15% of her body weight in a short time (e.g. 4-6 weeks) without having BMI fall below 17.5, consistent with the International Statistical Classification of Diseases and Related Health Problems 10 (ICD-10) diagnosis of atypical AN (254). Recovery was defined as having a BMI ≥ 18.5, being weight stable for at least 3 months, absence of binging and purging, absence of another eating

disorder, and exercise not exceeding that recommended by the United States Department of Agriculture 2005 Dietary Guidelines (255). Except for 1 RAN and 1 C subject, all subjects had regular menses. The RAN subject was unable to menstruate without the use of oral contraceptives and chose not to take this medication and the C subject continually took oral contraceptives without taking a week of placebo to avoid menstruation.

The protocol used in this study was approved by the Rutgers University Institutional Review Board for the Protection of Human Subjects and informed written consent was obtained from all subjects.

Anthropometric Measurements

Height was measured to the nearest 0.1 cm using a stadiometer (Invicta Plastics Ltd., Oadby, UK) and weight was measured to the nearest 0.1 kg using a digital scale (Tanita BF 578, Tokyo, Japan). Each subject was measured wearing light clothing and socks (e.g. jeans and a t-shirt).

Body composition

Body composition and body fat distribution were measured using dual energy x-ray absorptiometry (DXA) (Lunar Prodigy Advanced DXA, GE-Lunar, Madison, WI with enCORE 2004 software version, 8.10.027). DXA has been shown to be an accurate method to measure body composition (302,303). The DXA was calibrated, using a phantom scan, according to the standard protocol recommended by the manufacturer. Each subject was positioned on the DXA using a standardized protocol for positioning of the head, torso and limbs and asked to remain still while a whole body scan was performed. The DXA software

automatically divides the body into segments and total body, extremity, trunk [from the shoulders to femoral neck (262-265)], android, and gynoid regions were used for our analyses. Total fat tissue was used for each region.

Statistical Analysis

Data were determined to be normally distributed, using a skewness test and the skewness calculated was less than twice its standard error. Student's t-test was used to determine if differences in baseline variables, body composition, and body fat distribution existed between RAN and C subjects. Effect size was calculated using Cohen's *d* and Pearson's *r* for outcome variables that were found to be statistically significantly different between groups. In accordance with previously established standards, an absolute value for Cohen's *d* of 0.50 was considered to have a medium effect and an absolute value Cohen's *d* of ≥ 0.80 a large effect (298).

Multiple linear regression analyses were used to determine if there was a relationship between fat mass or body fat distribution and recovery from AN. TrFM adjusted for FM (AdjTrFM) was constructed from the residuals of TrFM regressed on FM. The following regression analyses were conducted: 1a) FM = β + LBM + group, 1b) FM = β + LBM + group + height, and 1c) FM = β + LBM + group + height^2; 2) AdjTrFM = β + LBM + group + height^2 and 3) TrFM:FM = β + LBM + group + height^2. Effect size was calculated using Partial Eta Squared for independent variables that were statistically significant for each regression equation. All analyses were conducted using SPSS 14.0 for Windows (SPSS Inc, Chicago, IL) and a p-value ≤ 0.05 was considered statistically significant.

Results

Subject Characteristics

Clinical characteristics for RAN and C subjects are presented in **Table A-1**. Differences between the groups did not exist for height, weight, BMI, or age. The lowest BMI for the RAN subjects was significantly lower than that for the C subjects, (16.5 and 20.5, respectively ($p < 0.001$)). The average length of recovery for RAN subjects was 6.4 years.

Body composition characteristics are presented in **Table A-2a.** Total FM and LBM were similar between RAN and C subjects (FM was 17.4 kg and 19.5 kg and LBM was 40.4 kg and 40.7 kg, respectively). RAN subjects had a borderline significant lower bone mass, (2.4 kg vs. 2.6 kg, respectively ($p = 0.080$)). Cohen's *d* indicated a medium effect size (298).

Body Fat Distribution

Body fat distribution was also similar in RAN and C subjects. No differences existed in truncal, android, gynoid, or extremity fat (**Table A-2b**). Percent truncal fat was 27.2% and 30.5% respectively, (ns). Using multiple linear regression analysis, the relationship between group (RAN or C) and FM, AdjTrFM, and TrFM:FM was studied. There was no effect of group on FM **(Table A-3a-c)**. Including height or height^2 in the model did not improve the model ($R^2 = -0.025$ v. $R^2 = 0.011$ v. $R^2 = 0.011$). Group was not significantly correlated to AdjTrFM ($p = 0.655$); when including LMB and height^2 the model explained 2.0% of the variance of AdjTrFM **(Table A-4)**. Variance of TrFM:FM was also not explained by group ($p = 0.184$) **(Table A-5)**.

Discussion

While it is possible that over time FM redistribution may occur, body composition and body fat distribution have not been thoroughly studied in long-term recovery from AN. Therefore, we studied body fat distribution in RAN subjects. We found no differences in body composition or body fat distribution between RAN and C subjects, but as expected RAN subjects had a trend for lower bone mineral content.

In undernutrition, LBM tends to be spared at the expense of FM, but following undernutrition, FM is gained more rapidly than LBM (2-4,86,90-92,196). For example, males in the Minnesota Starvation Study lost more than 25% of total body weight and gained FM more quickly than LBM during re-feeding (86). Stunted children also gained more FM and less LBM than non-stunted peers during early adolescent growth, though FM did not differ between groups (196). Consistent with these data, during weight restoration from AN, persons gained more FM than LBM (2-4,90-92) and FM is lower or comparable to control subjects (2-4,90-92). In this study, we found that several years following weight regain FM and LBM do not differ between RAN and C participants.

Similar to body composition, it is possible that body fat distribution may change overtime. While it is known that TrFM is higher in both short-term recovery from AN (3,4,71) and in people who suffered from undernutrition in early childhood (5,6,304), little information is available on the long-term effects of undernutrition on body composition and body fat distribution in adolescence or adulthood. Despite similarities in FM between recently weight restored AN and

control subjects (3,4), recently weight restored AN subjects had significantly higher central adiposity than control subjects (2-4,71,72). This could place people with a history of AN at risk for chronic diseases as high central FM is associated with increased risk of metabolic syndrome, type 2 diabetes, and cardiovascular disease (87-89). For example, adults who were born small for gestational age (SGA) have high central adiposity (5,6) and are at increased risk for metabolic syndrome, type 2 diabetes, and cardiovascular disease (5,6,11-13). Therefore, if people in long-term recovery from AN are similar to other groups that experienced undernutrition and have increased TrFM compared to people with no history of an eating disorder, it is plausible that people with a history of AN could be at increased risk for chronic diseases.

While short-term data indicate that women with a history of AN could be at increased risk for chronic disease (3,4,71), our data indicate that RAN women are not likely at increased risk for chronic diseases. We found no differences in body fat distribution, FM and LBM in each DXA segment (trunk, extremity, android and gynoid), between RAN and C women. These data are consistent with a recent study that reported no differences in body fat distribution between persons with a history of AN (weight recovered for > 2 years by final measurement) and control subjects (96). Therefore, adolescents and adults that experience undernutrition have body compositions similar to persons who did not experience undernutrition if weight restoration is maintained for ≥ 2 years.

Metabolic Programming

In terms of “programming” of body composition, both the timing of undernutrition and the time following undernutrition may have important implications for body fat distribution and health. Persons born SGA and stunted children experience starvation during early childhood (5,9,178,305-307). It has been argued that metabolic programming could occur during this time, such that persons are more “thrifty” thereby storing more fat centrally (5,9,178,305-307), perhaps for easier use if undernutrition should reoccur. However, the same may not be true of undernutrition occurring following early childhood. Overweight and obese adults who lost and regained weight had increased percent FM, but did not have increased central adiposity (201). It has also been shown that weight cycling, weight loss followed by weight regain, is not associated with increased percent FM or increased central adiposity when comparing initial and final measurements (308). Similar to overweight and obese adults, persons with AN experienced weight loss following growth. Despite differences in the severity of weight loss between the overweight and obese adults in these studies and people with AN, it is still possible that the metabolic programming seen in persons born SGA and in stunted children may not occur in RAN women.

While it is possible that the metabolic programming hypothesized in persons born SGA and stunted children may not occur in persons recovering from AN, short-term recovery following AN is characterized by central adiposity exceeding control subjects (2-4,71,72). However, data from the study presented in this dissertation indicate that when recovery from AN reaches or exceeds 2

years, body fat distribution does not differ from C women. Based on these findings it seems that metabolic programming may not occur in long-term recovery from AN.

Limitations

There are several limitations to this study. First, RAN women were recruited based on a self-reported previous diagnosis of AN. Due to logistical constraints and Health Insurance Portability and Accountability Act regulations, confirmation of diagnosis was not obtained. However, our screening questionnaire assessed lifetime history of AN (69,170,243-253), all RAN participants met diagnostic criteria for AN 2 or more years before the study and no C participants met diagnostic criteria for AN. Further, there was a trend for our AN group to have lower bone mass, consistent with other studies conducted on persons with a history of AN (45-47). Second, as this was a cross-sectional study, we were unable to determine changes in FM and body fat distribution in RAN women over time. However, ours is one of only a few groups to study body composition in long-term recovery following AN (96). Third, it has been argued that the rate of weight gain may have implications on FM and body fat distribution (4). We do not have information regarding the rate of weight gain in our RAN subjects. While knowing the rate of weight regain could provide further information regarding successful treatment of AN, lack of this information does not detract from our findings.

Other Implications

Weight gain and increased FM are necessary for recovery from AN. A higher increase of FM during initial weight restoration from AN is predictive of a higher likelihood of remaining in recovery (301). However, additional treatment challenges arise from anxiety produced by feelings that FM is gained disproportionately in the central region (97). Despite this challenge and a lack of effective, evidence-based treatments for AN (309-311), many people are able to recover from AN (62,65-67,70). Data from this study indicate that if weight restoration persists for 2 or more years, body fat distribution does not differ from C subject. This information could potentially help clinicians in treating persons with AN and improve overall treatment outcome along with long-term health in this population.

Conclusion

In summary, the amount of time following undernutrition may have important effects on body fat distribution. FM and body fat distribution in RAN women are comparable to that of C women. This information could not only be helpful in treatment of this disorder, but highlights another importance of developing effective, evidence-based treatments for AN. While AN is associated with serious physiological complications, if a person is able to recover, based on body composition and body fat distribution RAN individuals not are at increased risk for chronic diseases.

Table A-1 Baseline Characteristics of Women Recovered from Anorexia Nervosa ≥ 2 Years (RAN) and Control (C) Participants[1]

VARIABLE	RAN	C	p-value
N	16	18	
Height (cm)	166.0 ± 6.6	166.6 ± 6.4	0.797
Weight (kg)	61.0 ± 8.1	63.5 ± 8.6	0.390
BMI	21.9 ± 2.2	22.8 ± 2.5	0.264
Age (yrs)	23.5 ± 4.9	24.0 ± 4.5	0.766
Lowest BMI	16.5 ± 2.6	20.5 ± 1.5	0.000[2]
Months in Recovery	77.1 ± 51.9	N/A	N/A
Race:			
Caucasian	14	13	0.285
African American	0	1	0.354
Asian	2	1	0.491
Hispanic	0	1	0.354
Middle Eastern	0	2	0.180

[1]μ ± Standard Deviation for all variables
[2]Cohen's *d* = -1.885; Pearson's *r* = -0.702

Table A-2a Body Composition in Women Recovered from Anorexia Nervosa ≥ 2 Years (RAN) and Control (C) Participants[1]

VARIABLE	RAN	C	p-value
N	16	18	
Fat Mass (kg)	17.4 ± 5.6	19.5 ± 5.4	0.277
Lean Body Mass (kg)	40.4 ± 5.6	40.7 ± 5.6	0.889
Bone Mineral Content (g)	2413.83 ±363.53	2639.09 ± 361.20	0.080[2]

[1]μ ± Standard Deviation for all variables
[2]Cohen's *d* = -0.621; Pearson's *r* = -0.305

Table A-2b Body Fat Distribution in Women Recovered from Anorexia Nervosa ≥ 2 Years (RAN) and Control (C) Participants[1]

VARIABLE	RAN	C	p-value
Trunk (kg)	7.2 ± 3.5	8.5 ± 2.9	0.243
Android (kg)	1.1 ± 0.5	1.2 ± 0.5	0.354
Gynoid (kg)	3.2 ± 0.8	3.5 ± 0.8	0.284
Legs (kg)	7.3 ± 1.7	7.6 ±1.8	0.559
Arms (kg)	2.3 ± 0.8	2.7 ±1.1	0.234
Total (%)	29.8 ± 7.2	32.1 ± 6.4	0.330
Truncal (%)	27.2 ± 10.1	30.5 ± 8.0	0.288
Android (%)	29.9 ± 11.2	32.3 ± 9.9	0.520
Gynoid (%)	35.9 ± 6.0	37.4 ± 5.9	0.473
Legs (%)	33.4 ± 5.4	34.9 ±5.5	0.624
Arms (%)	35.7 ± 8.4	37.2 ± 9.5	0.427

[1]μ ± Standard Deviation for all variables

Table A-3a Total Fat Mass in Women Recovered from Anorexia Nervosa ≥ 2 Years (RAN) and Control (C) Participants

Fat Mass (R^2 = -0.025)

VARIABLE	Coefficient	Standard Error	p-value
Constant	18.614	7.277	0.016
Group*	-2.083	1.191	0.286
Lean Body Mass (kg)	0.022	0.176	0.900

***C = 0; RAN = 1

Table A-3b Total Fat Mass in Women Recovered from Anorexia Nervosa ≥ 2 Years (RAN) and Control (C) Participants

Fat Mass (R^2 = 0.011)

VARIABLE	Coefficient	Standard Error	p-value
Constant	-21.711	28.598	0.454
Group*	-1.974	1.887	0.304
Lean Body Mass (kg)	-0.212	0.236	0.376
Height (cm)	0.299	0.206	0.156

***C = 0; RAN = 1

Table A-3C Total Fat Mass in Women Recovering from Anorexia Nervosa ≥ 2 Years (RAN) and Control (C) Participants

Fat Mass (R^2 = 0.011)

VARIABLE	Coefficient	Standard Error	p-value
Constant	3.063	12.868	0.813
Group*	-1.975	1.887	0.304
Lean Body Mass (kg)	-0.213	0.237	0.375
Height^2 (cm^2)	0.001	0.001	0.156

***C = 0; RAN = 1

Table A-4 Truncal Fat Mass Adjusted for Total Fat Mass (AdjTrFM) in Women Recovered from Anorexia Nervosa ≥ 2 Years (RAN) and Control (C) Participants

AdjTrFM (R^2 = 0.021)

VARIABLE	Coefficient	Standard Error	p-value
Constant	2.819	2.226	0.215
Group*	-0.147	0.326	0.655
Lean Body Mass (kg)	-0.048	0.041	0.252
Height^2 (cm^2)	$-3.0 * 10^{-5}$	0.000	0.780

***C** = 0; RAN = 1

Table A-5 Truncal Fat Mass (TrFM) : Total Fat Mass (FM) in Women Recovered from Anorexia Nervosa ≥ 2 Years (RAN) and Control (C) Participants

TrFM:FM (R^2 = 0.069)

VARIABLE	Coefficient	Standard Error	p-value
Constant	0.430	0.153	0.009
Group*	-0.031	0.022	0.184
Lean Body Mass(kg)	-0.005	0.003	0.072[1]
Height^2 (cm^2)	$7.71*10^{-6}$	0.000	0.307

***C = 0; RAN = 1**

[1]Partial Eta^2 = 0.104

SECTION B RESPONSE TO STRESS IN LONG-TERM RECOVERY FROM ANOREXIA NERVOSA

Abstract

Background: Cortisol, a stress hormone, is associated with central adiposity. Cortisol levels are higher in women with Anorexia Nervosa (AN) and in short-term recovery from AN compared to women without a history of eating disorders. This has been shown in AN and weight stable, recovering women using urinary free cortisol, serum cortisol and salivary cortisol in the morning, afternoon and evening. Perfectionism and body dissatisfaction are increased in AN and short-term recovery from AN. Both perfectionism and body dissatisfaction have been associated with increased cortisol levels. If cortisol levels remain higher in women in long-term recovery following AN this group could be at risk for increased gain of central fat mass. Little is known about cortisol levels and variables that could affect cortisol levels at baseline or under stress in long-term recovery following AN, the focus of this study.

Objective: The aim of this study was to examine the effects of long-term recovery from AN on stress response and cortisol levels.

Design: Using a cross-sectional design, 16 women recovering from AN for 2 or more years (RAN) and 18 control (C) women were studied. Baseline cortisol was measured using saliva samples collected by the subject at home on a weekday upon awakening, 15 and 30 minutes later, at 3pm and at bed time. The Trier Social Stress Test (TSST) was used to elicit a stress response. Cortisol and heart rate were measured at baseline and during stress. Perfectionism and body satisfaction were assessed using the Perfectionistic Self-Presentation Scale and

Body Shape Questionnaire-34, respectively. Dual energy x-ray absorptiometry was used to measure body composition.

Results: RAN and C women did not differ in age (23.5 v. 24.0 (p = 0.766), respectively) or body mass index (21.9 V. 22.8, (P = 0.264), respectively). Fat mass, lean body mass and body fat distribution also did not differ between RAN and C subjects. No differences were found between RAN and C subjects for cortisol levels at baseline (morning, afternoon, or evening) or under stress. Following stress area under the curve was similar for both groups (109 nM/min v. 101 nM/min (p = 0.847), respectively). Average heart rate was lower for RAN subjects during the TSST (p = 0.036); baseline heart rate the day prior to the study did not differ between groups (p = 0.229). Nondisclosure of imperfection was significantly higher (p = 0.043) and there was a trend for higher body dissatisfaction (p = 0.073) in RAN compared to C women.

Conclusion: Long-term recovery following AN was not associated with increased cortisol levels at baseline or under stress despite higher nondisclosure of imperfection and lower body satisfaction, factors known to increase cortisol levels. Further, RAN individuals do not have an increased response to stress compared to control subjects indicated by both heart rate and cortisol levels. Therefore, based on these findings RAN participants are not at risk for increased gain of central fat mass, due to psychological stress induced cortisol response.

Introduction

Anorexia Nervosa (AN) is a severe psychological disorder, affecting approximately 1% of the female population in westernized society (1,21-23). It is characterized by an extreme low body weight (body mass index (BMI) below 17.5) accompanied by denial of the seriousness of the low body weight, an intense fear of gaining weight and amenorrhea (20). Disturbances in cortisol are often noted in persons with AN (2,4,124,160-163). Serum, urinary free cortisol and salivary cortisol have all been shown to be elevated during AN (2,4,124,160-163). Since cortisol is associated with increased central fat deposition (73,74,77), increased cortisol levels could predispose this group to gain central fat mass (FM). Gain of central FM poses treatment challenges (97) and health risks (87-89).

Cortisol

Cortisol is a stress hormone that is released by the adrenal glands in response to physical or psychological stress and regulated by the hypothalamic-pituitary-adrenal (HPA) axis. Disturbances in any portion of the HPA axis are associated with increased or decreased cortisol levels (73,74,98). As cortisol can be a catabolic hormone, it is possible that increased cortisol levels in starvation are adaptive and important for survival (74). Cortisol has been associated with increased lipoprotein lipase (LPL) transcription, translation and activity (99). In addition, cortisol facilitates the release of energy to the body via protein catabolism, gluconeogenesis and lypolysis (103-113,312).

Cortisol follows a circadian or diurnal rhythm (280,313) and is generally highest in the morning, followed by mid-day surges and a decrease into the evening (114-117,313). It has been shown that time of day may have an effect on cortisol secretion in response to stress (127-130,136,137). For example, using the adrenocorticotropic hormone (ACTH) stimulation test, a test which stimulates the release of cortisol, cortisol secretion has been shown to be higher in the afternoon or evening than the morning (127,128). Meals are also associated with cortisol increases (77,131-134), but the time of day at which a meal is eaten also has an impact on the resulting cortisol secretion with more cortisol being secreted in response to an afternoon than an evening meal (136,137).

Cortisol and Caloric Restriction

Caloric restriction and cortisol levels have been studied in several different groups of women (2,4,124,156-163). Pre-fasting or pre-caloric restriction body weight may influence the cortisol response (2,4,124,156-163). Fasting results in increased serum cortisol levels in normal weight women (156,157), but not obese women (158,159). In AN, cortisol levels have consistently been shown to be higher than persons without a history of an eating disorder (2,4,124,160-163,165). However, the long-term effects of severe undernutrition on cortisol levels have not been studied.

Cortisol and Stress

High cortisol levels under stressful conditions and in a baseline state have been associated with central obesity (4,75,138,139). For example, Moyer et al.

showed that under psychological stress women with a higher waist-to-hip ratio had higher salivary cortisol levels (139). Among women who expressed feeling stress resulting from a mental stress test, cortisol secretion was higher in women with higher visceral adipose tissue (VAT) (138). In a clinical study, a correlation between abnormal morning salivary cortisol variability and insulin, glucose, triglycerides, blood pressure, and abdominal obesity, all factors associated with the metabolic syndrome, has also been found (75). The high cortisol levels seen in AN (2,4,124,160-163,165) in conjunction with stress, could predispose this group for a disproportionately increased central fat gain upon weight restoration.

Since higher morning and stress cortisol levels are associated with increased central obesity (73-75,77) it is important to study cortisol levels and factors that could affect cortisol levels in groups at risk for increased cortisol levels. Perfectionism and body dissatisfaction are prevalent in short and long-term recovery following AN (65,170,213,216,221,235) and that could be associated with stress and increased cortisol (222,223,241) in persons recovered from AN. Thus far studies have started to link recovery from AN with increased cortisol and central FM (4), but much remains unknown. Little information is available on baseline cortisol levels or cortisol levels under stress in long-term recovery from AN. Therefore the aim of this study was to evaluate cortisol levels in a baseline and stressed state and stress response in women with a history of AN who have been in recovery for 2 or more years (RAN) compared to women without a history of an eating disorder. We studied perfectionism and body satisfaction in order to determine the relationship between these potential

stressors, cortisol levels and recovery from AN. In addition, we explored the relationship between cortisol levels and body composition in RAN and control (C) women.

Subjects and Methods

Subjects

Sixteen women with a history of AN and in recovery for 2 or more years and 18 healthy Control women without a history of eating disorders were included in this study. Cortisol levels were measured for 14 RAN and 12 C women. All subjects were between 18 and 35 years old, had a BMI ≥ 18.5, were weight stable ± 2.2 kg for 3 months, non-smokers, free from chronic diseases (e.g. Diabetes, Cushing's disease, or Crohn's disease) and not taking any medications that are known to influence metabolism or body composition (e.g. diet pills, steroids, or beta blockers).

Subjects were recruited via flyer advertisements, email advertisements and in-class announcements at Rutgers University. To assess each subject's history of AN, a screening questionnaire consisting of psychological variables indicative of AN, lowest lifetime weight, and menstrual history was administered (69,170,243-253). All subjects met the *Diagnostic and Statistical Manual of Mental Disorders, IV edition* (DSM-IV) criteria for AN 2 or more years prior to initial screening (20) except for 3 subjects who received a diagnosis of AN following a loss of greater than 15% of her body weight in a short time (e.g. 4-6 weeks) without having BMI fall below 17.5, consistent with the International Statistical Classification of Diseases and Related Health Problems 10 (ICD-10)

diagnosis of atypical AN (254). Recovery was defined as having a BMI ≥ 18.5, being weight stable for at least 3 months, absence of binging and purging, absence of another eating disorder, and exercise not exceeding that recommended by the United States Department of Agriculture 2005 Dietary Guidelines (255). Except for 1 RAN and 1 C subject, all subjects had regular menses. The RAN subject was unable to menstruate without the use of oral contraceptives and chose not to take the medication and the C subject continually took oral contraceptives without taking the placebo to avoid menstruation.

The protocol used in this study was approved by the Rutgers University Institutional Review Board for the Protection of Human Subjects and informed written consent was obtained from all subjects.

Anthropometric Measurements

Height was measured to the nearest 0.1 cm using a stadiometer (Invicta Plastics Ltd., Oadby, UK) and weight was measured to the nearest 0.1 kg using a digital scale (Tanita BF 578, Tokyo, Japan). Each subject was measured wearing light clothing and socks (e.g. jeans and a t-shirt). Heart rate was taken 3 times, while the subject was seated, 3 minutes apart in the non-dominant arm using an auto inflate blood pressure cuff (Omron HEM 705 CPN, Bannockburn, IL).

Baseline Cortisol Collection Protocol

Baseline salivary cortisol samples were collected at home by the subjects, thereby allowing researchers to obtain cortisol levels at various times of an

unremarkable day without intrusion from researchers, which may have increased cortisol levels. Salivary samples have been shown to be a valid method by which to collect cortisol (8,266). Each subject was asked to collect five saliva samples by chewing on cotton until the cotton was thoroughly saturated and return it to one of five salivettes, at home, the day prior to the study upon awakening, 15 and 30 minutes later, at 3pm and at bed time. To ensure consistency with cortisol levels, all samples were collected on a weekday when the subject was not under any unusual stress and all subjects were measured during the luteal phase of the menstrual cycle, which has been shown to be associated with the highest cortisol levels and has been utilized by other studies (156,259-261). Subjects were told not to consume alcohol for 12 hours prior to taking the first sample. Samples were returned to the researcher and frozen at -20^{o}C until analyzed (8,266).

Trier Social Stress Test

While many studies have utilized the stress of a meal for a stress test, (75,133-135) this is a physical stress and has higher variation in response to time of day compared to the Trier Social Stress Test (TSST) (136,137,274). In women, mental stress has been shown to produce a urinary or salivary cortisol stress response (139,271-273). Therefore, in order to study the relationship between long-term recovery from AN and mental stress, the TSST was used. Subjects were told to fast for 90 minutes before arriving for the measurement. Briefly, subjects rested for 15 minutes and then a saliva cortisol sample was collected by having the subject chew on a 1 inch piece of cotton rope. The subject was asked to rate on a scale from 1-10)1 = no stress and 10 = the

highest stress imaginable) how much mental stress she was experiencing. A heart rate monitor (Polar FS3, Kempele, Finland) was then fitted to each subject according to manufacturer's instructions and another cortisol sample and stress rating was collected.

For the oral portions of the TSST, each subject was asked to a give mock job interview for 5 minutes and then perform oral, mathematical calculations for an additional 5 minutes. Both oral portions of the TSST were conducted in front of 2 researchers unknown to the subject. Saliva samples, heart rate, and stress level were collected immediately following the TSST, and 10, 20, 30, and 45 minutes following the TSST (8,259,276,279,282-284). In addition, 13 minutes following the TSST, each subject stood for 12 minutes to measure recovery heart rate. Since cortisol follows a circadian rhythm (280), each TSST was conducted between 1 and 5pm. Due to scheduling needs of the subjects; subjects were scheduled at approximately 1:30pm, 3:00pm or 4:30pm.

Cortisol Analysis

Cortisol samples were centrifuged and analyzed using the Expanded Range High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit (Salimetrics, LLC, State College, PA) according to the manufacturer's instructions (266,285-288). Calculations for cortisol levels were made using the following equation provided by Salimetrics, LLC: 10^(y = mx + b); y is percent bound (B/BO,), b is the intercept, and m is the slope, x (Log of Conc.) = (y - b)/m.

Body Composition

Body composition and body fat distribution were measured using dual energy x-ray absorptiometry (DXA) (GE-Lunar, Madison, WI with enCORE 2004 software, version 8.10.027). The DXA was calibrated, using a phantom scan according to standard protocol recommended by the manufacturer. Each subject was positioned on the DXA using a standardized protocol for positioning of the head, torso and limbs and asked to remain still while a whole body scan was performed. Total tissue fat was used for each region and the truncal area was defined from the shoulders to the femoral neck (262-265).

Perfectionistic Self-Presentation Scale and Body Shape Questionnaire-34

Subjects completed the Perfectionistic Self-Presentation Scale (PSPS), a tool designed to measure perfectionism, and the Body Shape Questionnarie-34 (BSQ), a questionnaire designed to measure body satisfaction. Both tools have been shown to be valid, yield internally consistent results, results consistent with other similar measures, have been previously used in eating disorder populations, have reliability and test re-test reliability (219,236-238,295). Overall perfectionism and the three subscales of the PSPS: perfectionstic self-promotion, nondisplay of imperfection, and nondisclosure of imperfection were also studied (213,217-219).

The BSQ has been shown to be consistent with other scales that measure body satisfaction, have good test re-test reliability and normative values have been established for a population similar to the control population utilized in this study (237). BSQ was utilized as a whole; in addition, questions 15 and 30 were

analyzed individually as these are indicative of body checking and body avoidance (296).

Statistical Analyses

All data were determined to be normally distributed using a skewness test when skewness was less than twice its standard error. Cortisol levels, except for the awake +30 cortisol level, were not normally distributed; therefore, each value was transformed to the natural log equivalent. The awake +30 cortisol level was normally distributed and therefore this value was used in regression analyses. One C subject was found to be an outlier falling more than two standard deviations from the mean for cortisol levels and removed from cortisol analyses.

Student's t-test was used to estimate group differences in baseline variables, individual time points for baseline and stress cortisol levels perceived stress, PSPS and BSQ. Cohen's *d* and Pearson's *r* were calculated for variables that were found to differ significantly between groups and an absolute value for Cohen's *d* of 0.50 was considered to have a medium effect and an absolute value for Cohen's *d* of ≥ 0.80 a large effect (298).

The following multiple linear regression was performed to determine in the effect of exercise and group on heart rate at baseline, for each TSST time point, average heart rate and highest: heart rate = β + group + hours of exercise. There was no interaction between group and hours of exercise. Rate of change for heart rate was calculated as follows: (Δ point B to A) / (Δ time B to A (minutes)). General linear model (GLM) repeated measures was performed to determine the relationship between change in heart and recovery from AN at

each time point. Each of the six TSST time points was entered as a within-subject variable and group was entered as the between subject variable. The same analyses were conducted for cortisol, however the natural log for each cortisol value was used in the GLM repeated measures and seven time points were used.

Multiple linear regression analyses were used to determine if use of oral contraceptives had an influence on cortisol levels at baseline or during stress or on area under the curve (AUC) and if time of day at which the TSST was conducted had an influence on cortisol levels during stress or AUC. Dummy variables were created for each of the 3 times at which TSSTs were conducted as follows: Dummy 1 = (1:30pm = 1 and 3pm and 4:30pm = 0); Dummy 2 = (3pm = 1 and 1:30pm and 4:30pm = 0) and Dummy 3 (4:30pm = 1 and 1:30pm and 3:30pm = 0). The following multiple linear regression analysis was performed for each cortisol sample obtained: 1) natural log cortisol or AUC = β + group + use of oral contraceptive drugs and the following multiple linear regression analysis was performed for each cortisol sample obtained during the TSST 1) natural log cortisol or AUC = β + group + dummy 1 + dummy 2 + dummy 3. Neither time at which TSST was conducted nor use of oral contraceptives had a significant influence on cortisol levels or AUC so all TSST time groups and oral contraceptive groups were analyzed together to allow for higher power to detect differences in outcome measures.

The following regression analyses were performed to determine the relationship between highest baseline cortisol and psychological variables that

could influence cortisol and truncal fat and highest baseline cortisol: 1) awake +30 = β + BSQ + PSPS + group and 2) percent TrFM = β + group + awake +30. Partial Eta Squared was calculated for all independent variables that were statistically significant.

In order to determine if there was a difference in the TSST stress response, AUC was calculated for cortisol stress response. AUC was calculated using the SPSS syntax for trapezoidal factorial approach; $AUC_K = AUC_{Ki...Kn}$ + (time (TSST time point (1 - 7)) – lag time)*(lag cortisol + cortisol (TSST time point 1 - 7))/2 (222,274,299). AUC was not normally distributed and transformed into its natural log equivalent. ANOVA was utilized to determine the relationship between cortisol stress response and recovery from AN. The following regression equation was performed to determine the relationship between truncal fat and cortisol stress response: percent TrFM = β + group + AUC. Partial Eta Squared was calculated for outcome variables that differed significantly between groups. All analyses were conducted using SPSS 14.0 for Windows (SPSS Inc, Chicago, IL) and a p-value ≤ 0.05 was considered statistically significant.

Results

Subject Characteristics

Clinical characteristics for RAN and C subjects are presented in **Table B-1**. There were no statistical differences between the groups for hours of exercise per week, height, weight, FM, percent TrFM, BMI, or age. The lowest BMI for the RAN subjects was significantly lower than that for the C subjects, (16.5 and 20.5

($p < 0.001$), respectively). Average length of recovery for RAN subjects was 6.4 years.

Baseline Characteristics

Use of oral contraceptive drugs did not influence cortisol levels (data not shown). Baseline cortisol levels and heart rate did not differ between groups **(Table B-2a and Table B-2b).** There was significantly higher nondisclosure of imperfection ($p = 0.043$) and a trend for higher body dissatisfaction ($p = 0.073$), but not overall perfectionism ($p = 0.545$) in the RAN group **(Table B-2b)**. Cohen's *d* indicated a medium effect size (298) for both non-disclosure of imperfection and body dissatisfaction **(Table B-2b)**. Using multiple linear regression analysis, no significant relationship was found between the awake +30 cortisol level (the highest baseline cortisol level) and group, independent of perfectionism or body satisfaction **(Table B-3).**

TSST

There were no differences between RAN and C subjects for perceived stress (data not shown). Average heart rate over the course of the TSST was significantly lower in RAN compared to C subjects when controlling for hours of exercise per week (77 v. 89 ($p = 0.036$), respectively) **(Table B-4)**, and RAN subjects had a trend for significantly lower heart rates at several time points (before TSST, ($p \leq 0.051$); end TSST ($p \leq 0.062$); and TSST +30 ($p \leq 0.082$) **(Figure B-1)** and based on Partial Eta Squared, group explained 10%-12% of the variance in these heart rates. High heart rate did not differ between groups (183 v. 168 ($p = 0.585$), respectively) **(Table B-2b)**. Heart rate increased significantly

during the course of the TSST in both groups based on Pillai's Trace (p = 0.000), but the magnitude of the increase did not differ between groups (p = 0.950) **(Table B-5a).** There were no differences in rate of change for heart rate during the TSST (**Table B-5b; Figure B-1)**.

Time of day at which the TSST was performed did not have a significant influence on cortisol levels or cortisol stress response nor did use of oral contraceptives (data not shown). Cortisol levels at each time point did not differ between groups **(Table B-2a)**. Using GLM repeated measures, cortisol changed significantly during the course of the TSST (p = 0.000), but the change in cortisol did not differ by group (p = 0.266). Cortisol significantly increased from both the nadir and the arrival on the day of the TSST to the TSST + 10 minute time point for both groups **(Table B-5a)**, but the magnitude of the increase did not differ by groups (nadir: p = 0.933 and arrival: p = 0.668)**.** Cortisol also significantly decreased from the TSST + 10 minute time point to the TSST + 45 minute time point **(Table B-5a)** and the change in cortisol levels between measurements did not differ by group (p = 0.364). Rate of change for cortisol levels also did not differ between groups **(Table B-5c)**. There were no differences for change in cortisol for the nadir to arrival for the TSST between the groups studied (0.49 nM v. 0.27nM (p = 0.408), respectively).

Neither the AUC (109 nM/min v. 101 nM/min (p = 0.847), respectively) **(Figure B-2)** nor the curve resulting from cortisol levels (nM) v. time (min) differed between the RAN and C subjects. No relationship was found between stress response, recovery from AN, perfectionism or body satisfaction **(Table B-**

6a) and cortisol response to stress and recovery from AN were not predictive of percent TrFM **(Table B-6b)**.

Discussion

Since cortisol is a stress hormone, it is possible that the stress of experiencing AN may be associated with long-term alterations in cortisol secretions from the HPA axis in a baseline state or stressed state. High perfectionism and low body satisfaction are also two factors that could be associated with high cortisol in RAN individuals (65,170,213,216,221-223,235,241). As cortisol is also related to increased central adiposity (87-89,138) it is important to determine factors that may increase cortisol and the effects of cortisol on body composition in recovery from AN due to the negative health implication of large central adiposity. Thus, given that cortisol levels and stress response have not been thoroughly studied in long-term recovery following AN, we studied cortisol levels in both baseline and stressed states in RAN and C women. We found that RAN subjects did not have higher cortisol levels or an increased stress response and did not have higher percent TrFM than C subjects, despite differences in perfectionism and body satisfaction.

Cortisol in Anorexia Nervosa and Short-Term Recovery

Increased cortisol levels have been reported in people with AN using several different techniques including 24 hour urinary free cortisol, plasma cortisol, and salivary cortisol (2,4,124,160-163,165). For example, dos Santos et al. found salivary cortisol to be higher in AN than control subjects at 9am, 5pm and 11pm (124). Furthermore, cortisol levels in persons with AN who exercise

more and therefore are under more physical stress, are higher than persons who exercise less and are under less physical stress (314). Thus cortisol levels are altered during starvation.

Cortisol levels also remain high in re-feeding and short-term recovery from AN (2,4,124,160,161). Mayer et al. studied women with AN, and shortly after re-feeding from AN, and found that serum cortisol levels were higher than control participants both during and shortly following AN (4). In addition, women in short-term recovery from AN had higher TrFM, intramuscular adipose tissue and VAT compared to control women (4). Grinspoon et al. also reported that cortisol levels were higher in subjects with AN and cortisol and the ratio of percent TrFM to percent extremity fat were increased in short-term recovery from AN compared to control subjects (2). Further, higher urinary free cortisol was positively associated with increased TrFM gain during weight regain from AN (2). Still, neither study assessed cortisol levels under stress or in women in long-term recovery from AN.

Cortisol stress response may be altered in recovery from AN. While cortisol generally increases following a meal, there is some indication that cortisol does not increase in response to the stress of a meal in recovery following AN (170), suggesting possible alterations in cortisol secretion following AN. Therefore, it is important to determine if baseline and stress cortisol levels are higher in RAN than control subjects as high cortisol levels are associated with increased central adiposity (87-89,138).

Data from our study support that RAN subjects have cortisol levels that differ from persons with AN and in short-term recovery from AN (2,4). RAN subjects had cortisol levels that were comparable to C subjects. Further, percent TrFM and the relationship between cortisol and percent TrFM did not differ between groups. Therefore data from the study presented here suggests that recovery from AN could be associated with unremarkable body fat distribution and recovery of cortisol levels to normal physiological ranges (118,274).

Psychological Variables that Influence Cortisol

Perfectionism and body dissatisfaction are associated with increased cortisol levels (222,223,241). For example, using the TSST, men with higher perfectionism have been shown to have higher cortisol stress response indicated by AUC (222). A study of college students reported that, women with higher body dissatisfaction have higher afternoon cortisol levels than women with lower body dissatisfaction (241). Both perfectionism and body dissatisfaction remain high in recovery from AN (65,170,213,216,221,235) and therefore could contribute to increased cortisol levels.

In our sample, we found that overall perfectionism was not increased in RAN subjects, but RAN subjects scored higher on nondisclosure of imperfection indicating RAN women had a higher desire not to tell others of personal imperfections. We also found a trend for higher body dissatisfaction in RAN women. Despite higher nondisclosure of imperfection and a trend for greater body dissatisfaction in RAN subject, cortisol levels were not increased in RAN

subjects. Therefore, perfectionism and body satisfaction may have a different effect on cortisol levels in RAN.

The relationship between perfectionism and cortisol and body satisfaction and cortisol could differ in RAN women for several reasons. For example, it is possible that in this sample, differences between groups for nondisclosure of imperfection and body satisfaction were not robust enough to result in differences in cortisol levels. It is also possible that perfectionism and body satisfaction are lower now than during AN in our RAN group. If this was the case, the body of a RAN individual may not respond to the stress of the perfectionism and body dissatisfaction and therefore cortisol may not be higher than C women.

Stress Response in Long-Term Recovery from Anorexia Nervosa

Some aspects of stress response may be altered in RAN women, but cortisol stress response did not differ from control women. Cortisol levels did not differ between RAN and C participants at any individual time point. While cortisol significantly increased and decreased over the course of the TSST, the increase and decease did not differ between RAN and C participants. Further, RAN and C women had comparable AUC values for the TSST indicating a similar response to stress. While cortisol levels, AUC, and rate of change for cortisol levels did not differ between groups, RAN women had significantly lower average heart rate over the course of the TSST, even when controlling for hours of exercise per week. Hence, while cortisol levels and overall cortisol response to stress may not be altered, subtle alterations in overall stress response may occur.

There are several possible reasons that difference in overall stress response may occur. First, the RAN group may have been less stressed than the C group as indicated by lower heart rate over the course of the TSST. However, based on the subjective stress level, highest heart rate and AUC for cortisol the groups seemed to experience similar stress. Second, the RAN group may have experienced the same magnitude of stress, but for a shorter period of time, which could result in the lower heart rate over the course of the TSST, but not a lower value for the highest heart rate. Third, since AN puts so much stress on the body, the subjective perceived stress and the body's physiological response to stress could differ. It is possible that the body responds to stress as strongly and rapidly as persons without a history of an eating disorder, but also has an ability to rapidly decrease some aspects of stress response, such as heart rate, if the stress is not severe.

Possible Implications

Cortisol, in both a morning baseline state and a stressed state, has been associated with increased central adiposity (73-75,77). Also, high cortisol levels and TrFM have been found in persons in short-term recovery following AN (2,4,124,160,161). Thus, it is possible that persons in long-term recovery following AN also have increased cortisol and increased TrFM, factors associated with increased risk for chronic diseases (75,77,87-89,120). Data from this study indicates that RAN women are similar to control women as neither cortisol levels nor percent TrFM were higher in RAN compared to C participants. Furthermore, cortisol levels were not associated with percent TrFM in RAN or C

women. Based on our data, it does not seem that RAN individuals are at increased risk for central fat gain.

Limitations

There are several limitations to this study. First, RAN women were recruited based on a self-reported previous diagnosis of AN. Due to logistical constraints and Health Insurance Portability and Accountability Act regulations, confirmation of diagnosis was not obtained. However, our screening questionnaire assessed lifetime history of AN similar to those used elsewhere (69,170,243-253), all RAN subject met diagnostic criteria for AN 2 or more years before the study and no C volunteers met diagnostic criteria for AN.

Second, while we have heart rate data at given time points throughout the study, we do not have continuous data. Our data includes a measurement of the highest heart rate during the TSST, but we do not know the time at which this occurred and therefore cannot correlate this to the corresponding cortisol sample. Future studies should gather continuous data for heart rate during the TSST.

Third, subjects were asked to perform at home saliva collection on an unremarkable weekday when each participant did not have tests or papers to remove additional sources of variation. However, it is possible that the participants did not adhere to this instruction. Also, as we were studying a sample of college women, there was variation in the time at which the subjects woke up and went to bed, factors that could have influenced the cortisol levels obtained. Furthermore, while the first sample taken was in a fasted state, we do

not have information on when each subject ate relative to collecting cortisol sample, and the timing of a meal could have influenced cortisol levels. Thus, future studies should take cortisol samples at the same times for all subjects and at the same time interval following a meal.

Fourth, participants were instructed not to engage in vigorous physical activity the day prior to the study, but we were unable to assess if this instruction was followed; this could have been an additional source of variability on cortisol levels (314-318). Furthermore, collecting additional at home cortisol samples would provide more information about circadian rhythm for each individual. In addition, some of the women were using oral contraceptives which could have contributed to differences in cortisol level (259), but we did not find differences between women who were and were not using oral contraceptives.

Finally, while all TSSTs were conducted in a 4 hour time block it would be ideal to have all subjects begin the TSST at the same time. However this can be controlled for statistically as Bjorntorp et al. used the fuzzy set theory (319), Rosmond et al. used statistical weighting (131) while other groups have used methods similar to those utilized here to control for time of TSST (274,281). Further, the TSST has been shown to be robust enough to produce a cortisol response to stress, even in the afternoon (222,274-276), and we did not find a statistical difference between TSST time groups. Future studies should also examine cortisol levels for a longer period of time following the TSST as cortisol levels in our study had not returned to the values of the initial cortisol sample 45 minutes following the TSST.

Conclusion

Overall, cortisol levels, cortisol response to stress did not differ in RAN and C subjects. In addition, there were no statistically significant relationships between cortisol and body composition. While RAN subjects had significantly higher nondisclosure of imperfection and there was a trend for body satisfaction to be significantly lower, which could have lead to increased cortisol levels in RAN women, there was no difference in cortisol levels between RAN and C women. Therefore, based on these data cortisol and cortisol response to stress do not differ between RAN and C women.

Table B-1 Baseline Characteristics of Women Recovered from Anorexia Nervosa ≥ 2 Years (RAN) and Control (C) Participants[1]

VARIABLE	RAN	C	p-value
N	16	18	
Height (cm)	166.0 ± 6.6	166.6 ± 6.4	0.797
Weight (kg)	60.2 ± 8.0	62.8 ± 8.3	0.364
Fat Mass (kg)	17.4 ± 5.6	19.5 ± 5.4	0.277
Truncal (%)	27.3 ± 9.8	29.6 ± 8.9	0.463
BMI	21.9 ± 2.2	22.8 ± 2.5	0.264
Age (yrs)	23.5 ± 4.9	24.0 ± 4.5	0.766
Hours of Exercise per Week	5.6 ± 5.5	4.6 ±4.4	0.574
Lowest BMI	16.5 ± 2.6	20.5 ± 1.5	0.000[2]
Months in Recovery	77.1 ± 51.9	N/A	N/A
Race:			
Caucasian	14	13	0.285
African American	0	1	0.354
Asian	2	1	0.491
Hispanic	0	1	0.354
Middle Eastern	0	2	0.180

[1]μ ± Standard Deviation for all variables
[2]Cohen's *d* = -1.885; Pearson's *r* = -0.702

Table B-2a Cortisol Levels of Women Recovered from Anorexia Nervosa ≥ 2 Years (RAN) and Control (C) Participants[1]

VARIABLE	RAN	C	p-value
Awake (nM)[2]	12.6 ± 11.2	11.8 ± 8.7	0.847
Awake +15 (nM)[3]	26.9 ± 26.6	21.2 ± 10.5	0.510
Awake +30 (nM)[3]	29.4 ± 23.5	33.2 ± 20.3	0.675
3 pm (nM)[3]	8.6 ± 13.1	2.0 ± 2.4	0.112
Bed (nM)[2]	3.2 ± 5.9	2.9 ± 3.48	0.829
Arrival (nM)[3]	5.5 ± 4.8	4.8 ± 5.5	0.734
Prior to TSST(nM)[3]	7.9 ± 5.4	5.5 ± 6.09	0.308
Finish TSST (nM)[3]	21.3 ± 17.5	14.3 ± 10.3	0.253
TSST +10 (nM)[3]	22.2 ± 25.2	21.8 ± 21.3	0.966
TSST +20 (nM)[3]	17.3 ± 24.2	19.3 ± 20.9	0.826
TSST +30 (nM)[3]	11.0 ± 16.4	14.1 ± 14.0	0.614
TSST +45 (nM)[3]	11.9 ± 26.0	13.0 ± 17.0	0.908

[1] μ ± Standard Deviation for all variables
[2] N = 13 RAN and 11 C
[3] N = 14 RAN and 11 C

Table B-2b Heart Rate and Psychological Characteristics of Women Recovered from Anorexia Nervosa ≥ 2 Years (RAN) and Control (C) Participants[1]

VARIABLE	RAN	C	p-value
Baseline Heart Rate (beats per minute)[2]	73 ± 11	79 ± 10	0.229
Average Heart Rate (beats per minute)[3]	77 ± 10	89 ± 16	0.036[9]
Highest Heart Rate (beats per minute)[3]	183 ± 54	168 ± 53	0.585
Recovery Heart Rate (beats per minute)[3]	79 ± 14	88 ± 17	0.157
PSPS[5]	104 ± 30	99 ± 20	0.545
PSPS Perfectionstic Self-Promotion Scale	40 ± 14	38 ± 8	0.582
PSPS of Nondisplay Imperfection Scale	40 ± 13	41 ± 11	0.757
PSPS Nondisclosure of Imperfection Scale	24 ± 7	20 ± 5	0.043[10]
Body Shape Questionnaire BSQ[6]	100 ± 33	81 ± 28	0.073[11]
BSQ Question 15[7]	3 ± 1	3 ± 1	0.434
BSQ Question 30[8]	3 ± 2	3 ± 1	0.978

[1] μ ± Standard Deviation for all variables
[2] N = 16 RAN and 17 C
[3] N = 14 RAN and 18 C
[4] Controlling for hours of exercise per week
[5] Perfectionistic Self-Presentation Scale (PSPS)
[6] Body Shape Questionnaire (BSQ)
[7] Body Checking
[8] Body Avoidance
[9] Cohen's *d* = -0.899; Pearson's *r* = -0.388
[10] Cohen's *d* = 0.658; Pearson's *r* = 0.349
[11] Cohen's *d* = 0.621; Pearson's *r* = 0.312

Table B-3a Awake + 30 Cortisol in Women Recovered from Anorexia Nervosa ≥ 2 Years (RAN) and Control (C) Participants

Awake +30 (R^2 = -0.070)

VARIABLE	Coefficient	Standard Error	p-value
Constant	53.597	20.038	0.014
Group*	-0.127	9.968	0.990
PSPS	-0.179	0.171	0.308
BSQ	-0.050	0.143	0.727

***C = 0; RAN = 1

Table B-3b Percent Fat mass in Women Recovered from Anorexia Nervosa ≥ 2 Years (RAN) and Control (C) Participants

Percent Truncal Fat Mass (R^2 = 0.006)

VARIABLE	Coefficient	Standard Error	p-value
Constant	33.978	3.989	0.000
Group*	-4.607	3.718	0.228
Awake + 30 Cortisol (nM)	-0.076	0.086	0.387

***C = 0; RAN = 1

Table B-4 Heart Rate and Exercise of Women Recovered from Anorexia Nervosa ≥ 2 Years (RAN) and Control (C) Participants[1]

Average Heart Rate (R^2 = 0.426)

VARIABLE	Coefficient	Standard Error	p-value
Constant	99.213	3.236	0.000
Group*	-8.884	4.032	0.036[1]
Hours of Exercise per week	-1.678	0.408	0.000[2]

***C = 0; RAN = 1**

[1] Partial Eta^2 = 0.143

[2] Partial Eta^2 = 0.368

Table B-5a Changes in Heart Rate and Cortisol during the TSST in Women Recovered from Anorexia Nervosa ≥ 2 Years (RAN) and Control (C) Participants[1]

VARIABLE	RAN	p-value	C	p-value
Baseline Heart Rate and End of TSST (beats per minute)[2]	13.40 ± 10.50	0.000	18.50 ± 15.40[6]	0.000
Arrival Heart Rate and End of TSST (beats per minute)[3]	10.5 ± 8.80	0.001	12.6 0± 11.30[6]	0.000
End of TSST and TSST +10 (beats per minute)[3]	12.8 ± 8.20	0.000	-16.30 ± 12.20[6]	0.000
Nadir and TSST +10 (nM) [4]	19.12 ± 25.00	0.017	18.95 ± 22.23[6]	0.018
Arrival and TSST +10 (nM) [5]	16.64 ± 22.86	0.017	4.05 ± 5.47[6]	0.026
TSST +10 and TSST +45 (nM)[5]	10.27 ± 12.63	0.009	8.80 ± 10.44[6]	0.019

[1] μ ± Standard Deviation for all variables
[2] N = 14 RAN and 17 C
[3] N = 14 RAN and 18 C
[4] N = 13 RAN and 11 C
[5] N = 14 RAN and 11 C
[6] Did not differ from RAN subjects

Table B-5b Rate of Change for Heart Rate[1] between Trier Social Stress Test Time Points in Women Recovered from Anorexia Nervosa ≥ 2 Years (RAN) and Control (C) Participants[2, 3, 4]

VARIABLE	RAN	C
N	14	18
Equipment Placement and TSST End	0.50 ± 0.45	0.65 ± 0.63
TSST End and TSST +10 minutes	-1.28 ± 0.82	-1.63 ± 1.22
TSST +10 minutes and TSST +20 minutes	0.72 ± 0.56	0.78 ± 1.07
TSST +20 minutes and TSST +30 minutes	0.89 ± 0.59	-0.84 ± 0.96
TSST +30 minutes and TSST +45 minutes	0.13 ± 0.33	0.06 ± 0.45

[1] Beats/min for all
[2] μ ± Standard Deviation for Rate of change
[3] Pillai's Trace for change in Heart Rate 0.778; F = 18.187; df 5.00, 26.00; $p < 0.000$.
[4] Pillai's Trace for change in Heart Rate*group 0.041; F = 0.222; df 5.00, 26.00; $p < 0.950$.

Table B-5c Rate of Change for Cortisol[1] between Trier Social Stress Test Time Points in Women Recovered from Anorexia Nervosa ≥ 2 Years (RAN) and Control (C) Participants[2, 3, 4]

VARIABLE	RAN	C
N	14	11
Arrival and Equipment Placement	0.41 ± 0.80	0.11 ± 0.44
Equipment Placement and TSST End	0.62 ± 0.74	0.43 ± 0.31
TSST End and TSST +10 minutes	0.09 ± 1.38	0.75 ± 1.74
TSST +10 minutes and TSST +20 minutes	-0.49 ± 0.56	-0.24 ± 0.42
TSST +20 minutes and TSST +30 minutes	-0.63 ± 0.87	0.52 ± 1.06
TSST +30 minutes and TSST +45 minutes	0.06 ± 0.75	-0.08 ± 0.52

[1] nM/min for all
[2] μ ± Standard Deviation for Rate of change
[3]Pillai's Trace for change in Cortisol 0.856; F = 17.895; df 6.00, 18.00; p < 0.000.
[4]Pillai's Trace for change in Cortisol * group 0.319; F = 1.407; df 6.00, 18.00; P < 0.266.

Table B-6a Cortisol Stress Response in Women Recovered from Anorexia Nervosa ≥ 2 Years (RAN) and Control (C) Participants

Area Under the Curve (R^2 = -0.141)

VARIABLE	Coefficient	Standard Error	p-value
Constant	4.378	0.801	0.00
Group*	0.040	0.398	0.922
PSPS	-0.001	0.007	0.859
BSQ	0.000	0.006	0.956

***C = 0; RAN = 1

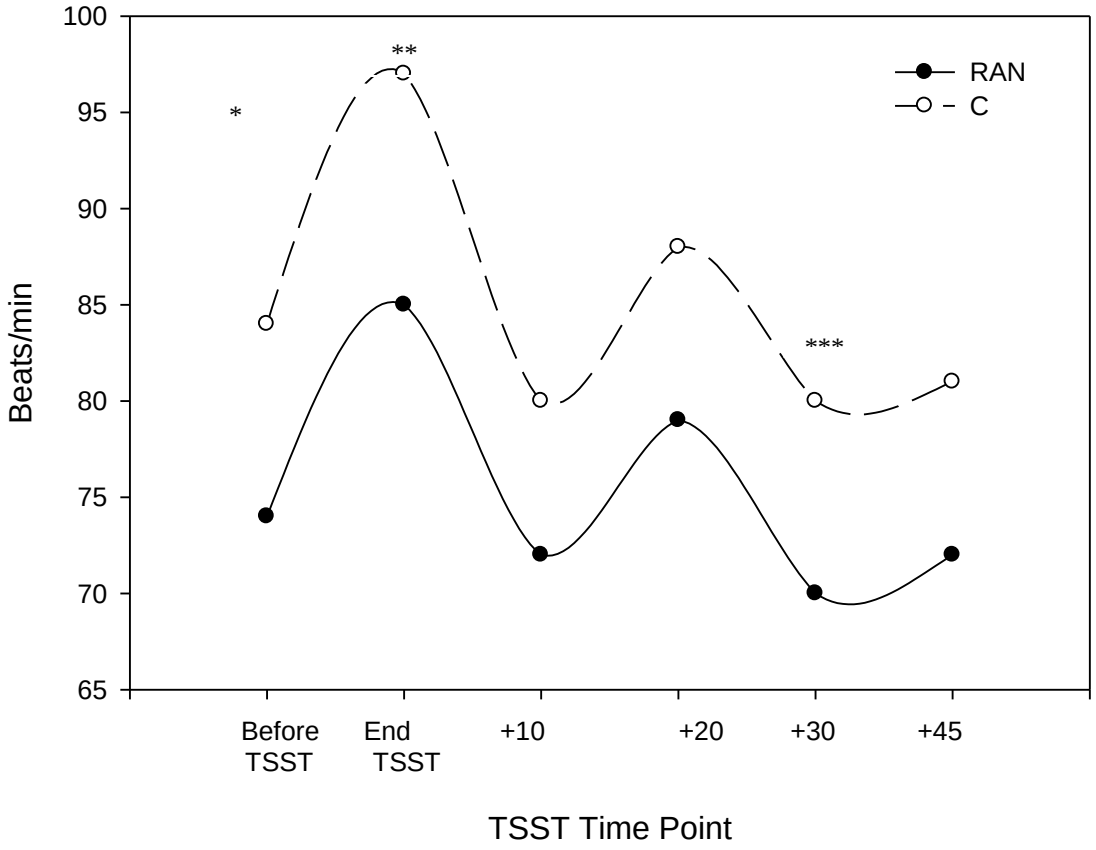
Table B-6b Body Fat Distribution and Cortisol Stress Response in Women Recovered from Anorexia Nervosa ≥ 2 Years (RAN) and Control (C) Participants

Percent Truncal Fat Mass (R^2 = -0.024)

VARIABLE	Coefficient	Standard Error	p-value
Constant	32.124	3.398	0.000
Group*	-4.262	3.760	0.269
Area Under the Curve	-0.007	0.019	0.726

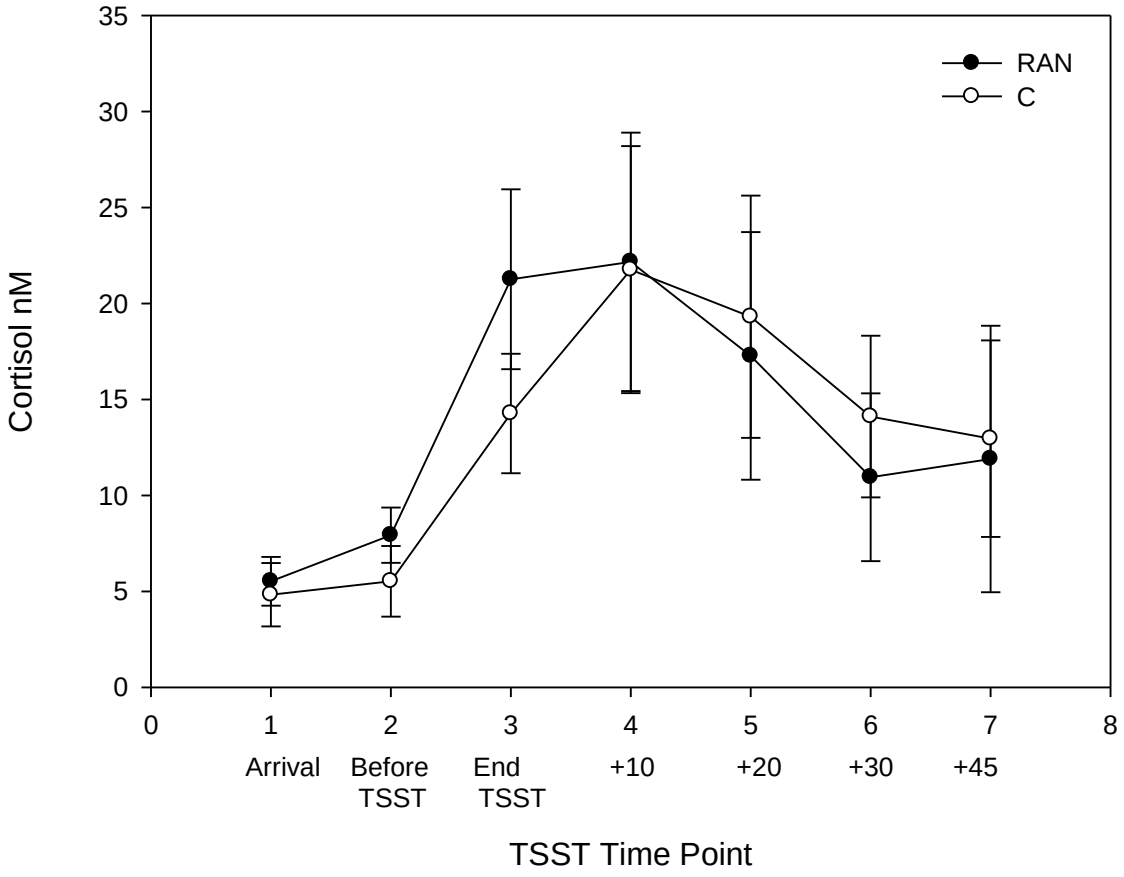
***C = 0; RAN = 1

Figure B-1 Heart Rate Change between Trier Social Stress Test (TSST) Time Points in Women Recovered from Anorexia Nervosa ≥ 2 Years (RAN) and Control (C) Participants[1]



*P ≤ 0.051; Partial Eta^2 = 0.125
**P ≤ 0.062; Partial Eta^2 = 0.115
***P ≤ 0.082; Partial Eta^2 = 0.101
[1]Controlling for hours of exercise per week

Figure B-2 Cortisol Change between Trier Social Stress Test (TSST) Time Points in Women Recovered from Anorexia Nervosa ≥ 2 Years (RAN) and Control (C) Participants



SECTION C INCREASED FAT METABOLISM IN LONG-TERM RECOVERY FROM ANOREXIA NERVOSA

Abstract

Background: It has been reported that resting energy expenditure (REE) is lower in individuals with anorexia nervosa (AN) compared to individuals without an eating disorder. Data on substrate oxidation in individuals with AN are inconsistent. Little information is available about the long-term effects of AN on REE and substrate oxidation.

Objective: The aim of this study was to determine if REE and substrate oxidation differ between persons in long-term recovery from AN (RAN) and control (C) subjects.

Design: Using a cross-sectional design, 16 women recovering from AN for 2 or more years and 17 control women were studied. REE and substrate oxidation was measured using indirect calorimetry and body composition was measured using dual energy x-ray absorptiometry.

Results: No differences were found between RAN and C subjects for REE (1270 ± 166 kcal/day and 1343 ± 126 kcal/day ($p = 0.162$), respectively) or REE adjusted for lean body mass (1273 ± 28 kcal/day v. 1340 ± 27 kcal/day ($p = 0.097$), respectively). RAN subjects had lower respiratory quotient (RQ) than C subjects (0.84 ± 0.05 and 0.89 ± 0.04, respectively) and differences remained when controlling for diet and body composition ($p \leq 0.014$).

Conclusion: In long-term recovery following AN, substrate oxidation is lower in RAN than C subjects, with no significant differences in REE. Further studies are needed to determine the mechanism and potential health impact of the lower RQ.

Introduction

Anorexia Nervosa (AN) is a severe psychological disorder associated with self-starvation and maintenance of a body mass index (BMI) at or below 17.5 (20). AN affects approximately 1% of the female population in Westernized societies (1,21-23). Generally, during starvation, the body preserves lean body mass (LBM), a major determinant of energy metabolism. Thus, alterations in energy metabolism may occur as a result of starvation and be a product of decreased overall body mass. However, effects of AN on resting energy expenditure (REE) and substrate oxidation in long-term recovery from AN are unknown and are the aims of this study.

In AN, REE has consistently been shown to be lower in AN than control subjects (93,179-183,187,320). Most studies, however, do not control for LBM, a major factor influencing REE. In one study that did adjust REE for LBM, REE was still found to be lower in the AN compared to the control group (183). In addition to low REE, substrate oxidation may be altered in AN, however findings in people with AN have been inconsistent (181,183,184,188,204-206). For example, antecedent diet is known to influence substrate oxidation (321) and it has been shown that macronutrient composition of diets differ between AN and control subjects (181,207,208), however diet was not controlled for in these studies. More importantly, even less is known about substrate oxidation during and following re-feeding. Since re-feeding may be associated with a shift in macronutrient composition, as well as increased caloric intake, substrate oxidation may also differ during and following re-feeding. Thus, these dietary

changes, in addition to changes in body composition, could influence energy metabolism.

To our knowledge, no studies of AN and energy metabolism have controlled for both body composition and macronutrient intake. In the current study, we study women recovering from AN for 2 or more years (RAN) to determine the long-term impact of AN on energy metabolism while controlling for body composition and dietary intake.

Subjects and Methods

Subjects

Sixteen women with a history of AN and in recovery for 2 or more years and 17 healthy control (C) women without a history of eating disorders were included in this study. All subjects were between 18 and 35 years of age, had a BMI ≥ 18.5, were weight stable ± 2.2 kg for 3 months, non-smokers, free from chronic diseases (e.g. Diabetes, Cushing's disease, or Crohn's disease) and not taking any medications that are known to influence metabolism or body composition (e.g. diet pills, steroids, or beta blockers).

Subjects were recruited via flyer advertisements, email advertisements and in-class announcements at Rutgers University. To assess each subject's history of AN, a screening questionnaire consisting of psychological variables indicative of AN, lowest lifetime weight, and menstrual history was administered (69,170,243-253). All subjects met the *Diagnostic and Statistical Manual of Mental Disorders, IV edition* (DSM-IV) criteria for AN 2 or more years prior to initial screening (20) except for 3 subjects who received a diagnosis of AN

following losing greater than 15% of her body weight in a short time (e.g. 4-6 weeks) without having BMI fall below 17.5, consistent with the International Statistical Classification of Diseases and Related Health Problems 10 (ICD-10) diagnosis of atypical AN (254). Recovery was defined as follows: BMI ≥ 18.5, weight stable for 3 months or greater, absence of binging and purging, absence of another eating disorder, and exercise not exceeding that recommended by the United States Department of Agriculture 2005 Dietary Guidelines (255). Except for 1 RAN and 1 C subject, all subjects had regular menses. The RAN subject was unable to menstruate without the use of oral contraceptives and chose not to take this medication and the C subject continually took oral contraceptives without taking the placebo pills to avoid menstruation.

The protocol used in this study was approved by the Rutgers University Institutional Review Board for the Protection of Human Subjects and informed written consent was obtained from all subjects.

Protocol

Screening questionnaires to assess history of eating disorders, recovery from eating disorders, and general health were completed for each subject. Height was measured to the nearest 0.1 cm using a stadiometer (Invicta Plastics Ltd., Oadby, UK) and weight was measured to the nearest 0.1 kg using a digital scale (Tanita BF 578, Tokyo, Japan) with each subject wearing light clothing and socks (e.g. jeans and a t-shirt). Height and weight measurements were taken two days prior to the study day to calculate caloric needs.

Diet

To ensure that measurements were not influenced by dietary consumption the day prior to the study, each subject was provided with all meals and snacks the day before measurements were taken. Macronutrient composition of the non-vegetarian diet was approximately 50% carbohydrate, 32% fat and 18% protein (food quotient (FQ) = 0.86) and the vegetarian diet 52% carbohydrate, 35% fat and 13% protein (FQ = 0.86). Estimation of caloric requirement was made using the Mifflin equation with an activity factor of 1.4 (moderately active) (289,290). Meals were provided by Brower Dining Hall at Rutgers, The State University of New Jersey. Subjects were also instructed not to engage in vigorous physical activity.

Two days prior to the measurements, each subject was given a list of food items and instructed to consume each item on the list and not to consume additional food or beverage items. Each subject was further instructed to cross off each item, as the item was consumed, and return the list to the researcher. Actual caloric consumption was calculated using the nutritional information provided by Brower Dining Hall and FQ was calculated using the following equation: FQ = (0.207 * %carbohydrate + 0.159 * %fat + 0.193 * %protein) / (0.207 * %carbohydrate + 0.226 * % protein + 0.243 * %fat) (293).

Indirect Calorimetry

Indirect calorimetry (IC) (VMax Spectra 29N, Sensormedics, Inc., Yorba Linda, CA) was used to measure REE and respiratory quotient (RQ) in the fasted state. Each subject was instructed to fast overnight and avoid any physical

activity prior to their morning appointment and scheduled shortly after waking. Upon arrival, each subject rested in a bed while the calorimeter was calibrated using a standard mix of carbon dioxide (4%) and oxygen (16%). Thereafter, a transparent hood was placed over the subject's head and measurements were conducted for 30 minutes, during which time subjects were instructed to lay quietly and avoid all types of motion, including fidgeting. The first 10 minutes of each measurement were not used to estimate the REE and RQ. REE was estimated by IC using the following equation REE (kcal) = 3.781 * O_2L + 1.237 * VCO_2L (294) and RQ was calculated by IC using the following equation: VCO_2 / VO_2.

Body composition

Body composition was measured using a dual energy x-ray absorptiometry (DXA) (GE-Lunar, Madison, WI with enCORE 2004 software version, 8.10.027). Each subject was positioned on the DXA using a standard protocol for positioning of the head, torso and limbs and instructed to remain still while a whole body scan was performed. Total tissue fat was used for each region and truncal fat was defined from the shoulder area to the femoral neck (262-265). The DXA was calibrated using a phantom scan, according to the standard protocol recommended by the manufacturer.

Statistical Analyses

Data were determined to be normally distributed, using a skewness test when skewness was less than twice its standard error. Student's t-test was used to determine group differences between RAN and C subjects for estimated REE,

actual REE, and REE per kg of LBM (REE:LBM) FQ and RQ. REE adjusted for LBM (REEadj) was calculated using a general linear model where REE was the dependent variable, LBM the covariate and group the fixed factor.

In order to determine if there was a relationship between energy metabolism and recovery from AN, multiple linear regression analyses were used. The following regression analyses were conducted: 1) REE = β + LBM + FM + group; 2) REE = β + LBM + group and 3) RQ = β + FQ + LBM + Fat Mass (FM) + group. There was no interaction between LBM and group. Effect size was calculated using Cohen's *d* and Pearson's *r* for student's t-test or Partial Eta squared for regression analysis. Effect size was calculated for outcome variables that differed significantly between groups. In accordance with previously established standards, an absolute value for Cohen's *d* of 0.50 was considered to have a medium effect and an absolute value for Cohen's *d* of ≥ 0.80 a large effect (298). All analyses were conducted using SPSS 14.0 for Windows (SPSS Inc, Chicago, IL) and a p-value < 0.05 was considered statistically significant.

Results

Subject Characteristics

Clinical characteristics for RAN and C subjects are presented in **Table C-1**. There were no statistical differences between groups for height, weight, BMI, age, FM or LBM. The lowest BMI for the RAN subjects was significantly lower than that for the C subjects, (16.5 and 20.5 ($p \leq 0.001$), respectively). Average length of recovery for RAN subjects was 6.4 years.

Energy Metabolism

Energy metabolism variables are presented in **Table C-2.** Estimated and measured REE did not differ between RAN and C women (estimated- 1371 kcal/day and 1413 kcal/day and measured- 1270 kcal/day and 1343 kcal/day, respectively) **(Table C-2)**. REEadj also did not differ between groups (1272 kcal/day and 1340 kcal/day, respectively) **(Table C-2)**. While REE was significantly over-predicted in both RAN and C subjects, the magnitude of the over-prediction did not differ between groups (data not shown). Using multiple linear regression, REE was not significantly related to group ($p \leq 0.190$), but was significantly related to lean body mass ($p \leq 0.000$) **(Table C-3)** and explained approximately 48% of variance in REE when FM was included in the model. The relationship between REE and LBM did not differ by group ($P \leq 0.097$) and is presented in **Figure C-1**.

RQ was significantly lower in RAN compared to C subjects (0.84 and 0.89 ($p \leq 0.004$), respectively) and Cohen's *d* (-1.10) indicated a large effect size (298). Using multiple linear regression analysis, RAN women had significantly lower RQ ($p \leq 0.014$) when controlling for FQ, FM and LBM and the model explained 20% of variance **(Table C-4)** and based on Partial Eta^2 group explained approximately 20% of the variance in terms of macronutrient breakdown. RAN women oxidized significantly more fat than C women (51% v. 36%, respectively ($p \leq 0.004$)), with Cohen's *d* indicating a large effect size (298).

Discussion

Energy metabolism has rarely, if ever, been studied in long-term recovery following AN. Thus far, the studies that have measured energy metabolism during AN have reported that REE is lower in AN than control subjects (93,179-184,187,320), but increases upon re-feeding (93,180,186-191). However, most of these studies did not adjust REE for LBM, a factor that could lead to conflicting results and conclusions. In addition, no studies that have measured substrate oxidation also controlled for antecedent diet (181,184,188,204-206), a major factor that influences substrate oxidation. Therefore, given these gaps in knowledge, and the fact that neither REE nor substrate oxidation have been thoroughly studied in long-term recovery following AN, the objective of our study was to measure REE and RQ in RAN subjects. Our primary result is that RAN subjects have REE and REEadj that do not differ from C subjects, but lower RQ values.

Resting Energy Expenditure

AN subjects are reported to have lower REE than control subjects (93,179-183,187,320). For example, it has been shown that in AN REE was significantly lower than control subjects (1171 kcal/day v. 1379 kcal/day, respectively ($p \leq 0.050$) (320), however this study did not control for body composition (320). In general, lower REE could be due to overall decreased body mass. While most studies do not adequately address this fact, one study that did adjust REE for LBM still found REE to be lower in the group with AN

compared to the control group (183), suggesting that REE was decreased in individuals with AN.

Changes in REE occur during re-feeding such that REE increases from AN to the re-feeding period (93,180,186-191) and, following re-feeding, may remain lower than control subject (183) or may be comparable to control subjects (184,320). Such changes in REE may be due to changes in body composition. Further studies are needed to determine the long-term impact of AN on REE while controlling for body composition.

While data on REE following starvation exist for other previously undernourished samples, the data are inconsistent (9,10,175-178). Previous studies have indicated higher, comparable and lower REE in persons that experienced undernutrition compared to peers that did not experience undernutrition leading to the conclusion that there are no differences in REE following undernutrition in utero or in early childhood (9,10,175-178). However, undernutrition at either of these points could be inherently different than undernutrition occurring later in life. Therefore, information learned from studying these groups may not be generalizable to a population that experienced undernutrition following puberty. Hence, further studies are needed to determine the long-term impact of undernutrition on REE.

In the study presented here, we found that REE and REEadj did not differ between RAN and C subjects. Thus, long-term recovery from AN is not associated with the low REE as observed in AN, information that may be useful in determining long-term caloric needs for this population.

Substrate Oxidation

It has been reported that substrate oxidation does not differ between AN and control subjects (181,184,204). Yet, it has also been reported that RQ (183,188), carbohydrate (188,206) and protein oxidation are higher (205,206) and fat oxidation lower (188,205,206) in AN compared to control subjects. However, it must be noted that previous dietary intake, an important determinant of RQ (321), was not reported. Therefore if the macronutrient composition of the diet the day before the study differed between the two groups, RQ and macronutrient oxidation would be expected to differ. One study did report the macronutrient content of the diet for the AN, but not control group (204) and two studies reported that food journals were maintained by subjects, but did not report the information (181,205). Mirsa, et al. reported differences in fat and carbohydrate intake between groups, but did not control for this when analyzing group differences in substrate oxidation (181). Therefore, many of the inconsistencies in previous studies could be due to dietary differences between groups and lack of statistical adjustment for diet.

While little is known about energy metabolism during and following AN, there are several factors that could influence RQ and REE during this period, including macronutrient intake. Macronutrient intake may differ in persons with AN, typically with higher percentage of total calories coming from carbohydrates and lower percentage coming from fat (181,207,208). Changes in dietary composition could influence RQ (321,322). Previously, it has been shown that carbohydrate and protein utilization increased and fat oxidation decreased during

the initial re-feeding period (188). In re-feeding, non-protein RQ is higher than that of AN and control subjects (204) with carbohydrate oxidation being higher and lipid oxidation lower during re-feeding compared to both AN and control subjects (204). Shortly following re-feeding, RQ did not differ between AN and control subjects (183,184). Further, fat metabolism has been shown to comprise a higher percent of REE and carbohydrate metabolism a lower percent of REE approximately 6 months following re-feeding (188). Such changes may or may not be accounted for by dietary differences.

In our study, when controlling for diet, we found that RAN subjects had lower RQ and higher fat oxidation than C subjects. The RQ values for C subjects were consistent with RQ values reported in other studies (183,184). The effects of undernutrition in AN are different than the effects of moderate weight loss in adults further illustrating the importance of studying the effects of undernutrition. For example, results from our study differ from results reported from a study on overweight or obese adults who lost and regained weight and had low fat oxidation (201). Also, obese persons who lose weight have a higher RQ than lean individuals of similar body mass index (202,203). Therefore, the effects of undernutrition on RQ differ from that of moderate weight loss. These differences could be due to the magnitude of the weight loss or perhaps genetic differences between the groups.

It would be expected that the RAN subjects would have lower FM since this group has a lower RQ (193,194). However, we found that FM and LBM do not differ between RAN and C subjects. Therefore, while alterations in energy

metabolism occur following undernutrition, we are unable to explain why these differences do not result in changes in body composition. Possible mechanisms contributing to a lower RQ in RAN women may include adiponectin or peptide YY (PYY). Both adiponectin and PYY have been shown to increase fat oxidation, which would result in lower RQ values (323-326). Adiponectin and PYY have been shown to be higher in both AN (327-334) and during recovery following AN (328,334). Nevertheless, upon weight restoration, adiponectin and PYY have also been shown not to differ between groups (331,335). Adiponectin levels have been shown to both increase (336) and decrease upon re-feeding following AN (328). While it is possible that adiponectin or PYY could be potential mechanisms responsible for lower RQ in RAN subjects in the current study, further research is needed.

Limitations

There are several limitations to this study. First, RAN subjects were recruited based on a self-reported previous diagnosis of AN. Many of our subjects were no longer in touch with treatment professionals as recovery was exceeding 2 years and researchers were unable to contact treatment professionals due to Health Insurance Portability and Accountability Act concerns. However, our screening questionnaire assessed lifetime history of AN (69,170,243-253), all RAN subject met diagnostic criteria for AN 2 or more years before the study and no C volunteers met diagnostic criteria for AN. In addition, our RAN volunteers had a trend for lower bone mass as would be expected (250,337-339). Second, as this was a cross-sectional study, we were unable to

determine changes in energy metabolism in RAN subjects over time. However, ours is one of only a few groups to study energy metabolism in long-term recovery from AN. Furthermore, this study controlled for body composition and diet, something lacking in the literature.

Conclusion

In conclusion, based on the results of this study, women who have been recovering from AN for two or more years, have a lower RQ, but similar REE compared to C women. These results remained when controlling for body composition and diet.

Table C-1 Baseline Characteristics of Women Recovered from Anorexia Nervosa ≥ 2 Years (RAN) and Control (C) Participants[1]

VARIABLE	RAN	C	p-value
N	16	17	
Height (cm)	166.0 ± 6.6	167.1 ± 6.2	0.629
Weight (kg)	60.2 ± 8.0	64.1 ± 8.5	0.294
Fat Mass (kg)	17.4 ± 5.6	19.2 ± 5.3	0.200
Lean Body Mass (kg)	40.4 ± 5.6	40.9 ± 5.3	0.836
Bone Mineral Content (g)	2413.8 ±363.5	2653.6 ± 366.9	0.069[2]
BMI	21.9 ± 2.2	22.9 ± 2.6	0.237
Age (yrs)	23.5 ± 4.9	24.1 ± 4.6	0.726
Lowest BMI	16.5 ± 2.6	20.5 ± 1.5	0.000[3]
Months in Recovery	77.1 ± 51.9	N/A	
Race:			
Caucasian	14	12	0.248
African American	0	1	0.349
Asian	2	1	0.524
Hispanic	0	1	0.340
Middle Eastern	0	2	0.167

[1]μ ± Standard Deviation for all variables
[2]Cohen's *d* = -0.657 Pearson's *r* = -0.321
[3]Cohen's *d* = -1.885 Pearson's *r* = -0.697

Table C-2 Energy Metabolism in Women Recovered from Anorexia Nervosa ≥ 2 Years (RAN) and Control (C) Participants[1]

VARIABLE	RAN	C	p-value
N	16	18	
Respiratory Quotient	0.84 ± 0.05	0.89 ± 0.04	0.004[5]
Percent Fat Oxidation	51.4 ± 15.5	36.6 ± 17.7	0.004[6]
Percent Carbohydrate Oxidation	48.6 ± 15.5	63.7 ± 17.7	0.004[7]
Food Quotient	0.86 ±0.01	0.85 ± 0.01	0.082
REE[2] Estimated (kcal/day)	1371 ± 121	1413 ± 126	0.354
REE Measured (kcal/day)	1270 ± 166	1343 ± 126	0.162
REE/LBM[3] (kg) (kcal/day per kg LBM)	31.5 ± 2.70	33.2 ± 3.34	0.125
REE adjusted for (LBM)	1273 ± 28[4]	1340 ± 27[4]	0.097

[1]μ ± Standard Deviation for all variables unless otherwise noted
[2]Resting Energy Expenditure (REE)
[3] Lean Body Mass (LBM)
[4]μ ± Standard Error
[5]Cohen's *d* = -1.10 Pearson's *r* = -0.484
[6] Cohen's *d* = 0.890 Pearson's *r* = 0.483
[7] Cohen's *d* = -0.908 Pearson's *r* =-0.483

Table C-3 Resting Energy Expenditure in Women Recovered from Anorexia Nervosa ≥ 2 Years (RAN) and Control (C) Participants

Resting Energy Expenditure (R^2 = 0.478)

VARABILE	Coefficient	Standard Error	p-value
Constant	523.254	157.635	0.002
Group*	-51.713	38.530	0.190
Lean Body Mass (kg)	17.252	3.999	0.000[1]
Fat Mass (kg)	5.791	3.550	0.114

*****C = 0; RAN = 1

[1] Partial Eta^2 = 0.471

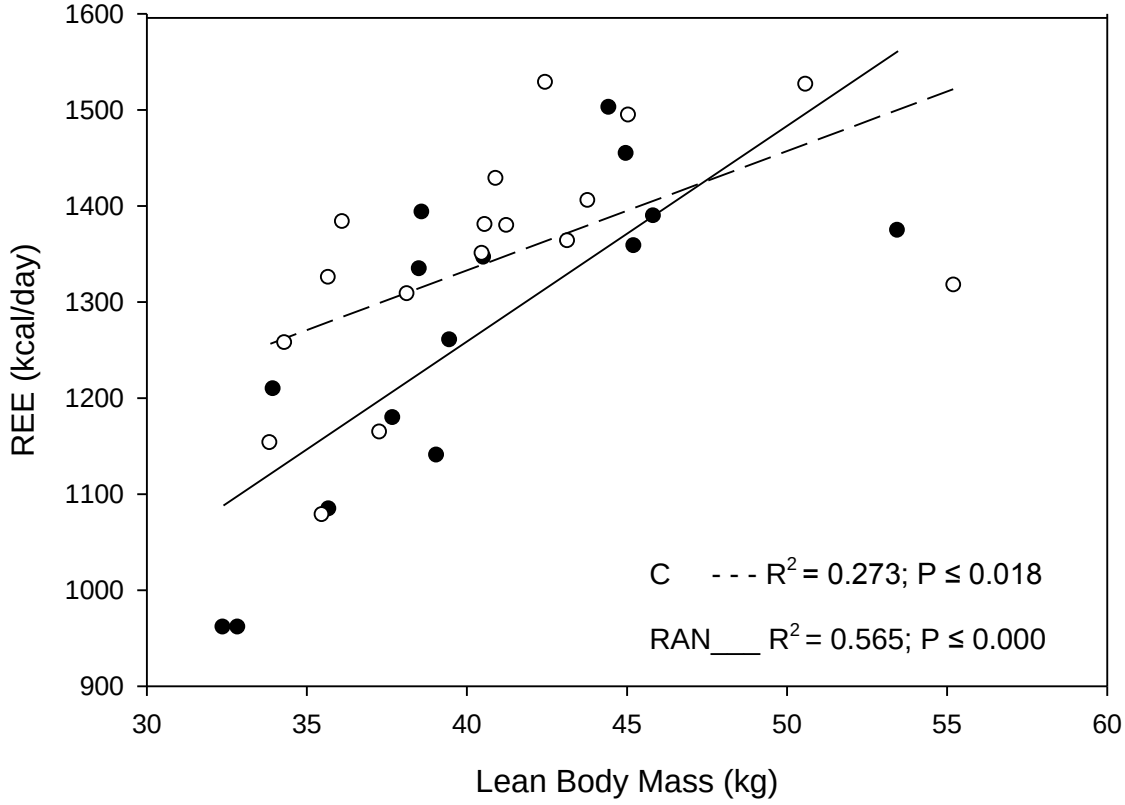
Table C-4 Respiratory Quotient in Women Recovered from Anorexia Nervosa ≥ 2 Years (RAN) and Control (C) Participants

Respiratory Quotient (R^2 = 0.199)

VARIABLE	Coefficient	Standard Error	p-value
Constant	0.585	0.550	0.196
Group*	-0.042	0.016	0.014
Food Quotient	0.257	0.635	0.688
Lean Body Mass (kg)	0.001	0.001	0.365
Fat Mass (kg)	0.002	0.001	0.233

***C = 0; RAN = 1

Figure C-1 Relationship between Resting Energy Expenditure and Lean Body Mass in Women Recovered from Anorexia Nervosa ≥ 2 Years (RAN●) and Control (C○) Participants



7 CONCLUSION

Based on the results from this study, it appears as though long-term recovery following anorexia nervosa (AN) is associated with low carbohydrate and high fat metabolism, but no other changes in metabolism or body composition. Research presented here in conjunction with earlier research conducted in short-term recovery following AN indicates that the time following undernutrition is important in metabolic recovery from starvation.

Previous studies on individuals in short-term recovery from AN (2-4,71,90-92,124,160,161,183) and people who experienced early childhood undernutrition (5,6,8-10,141-146,152,154,178,305-307) all suggest that there could be an increased risk for gain of central fat mass and associated chronic diseases following AN. However, in each segment of this research, women with a history of AN who have been in recovery for 2 or more years (RAN) showed metabolic profiles more similar to people without a history of an eating disorder (ED), than individuals that experienced undernutrition in early childhood and than individuals with AN or in short-term recovery following AN. This information further illustrates the importance of developing effective, evidence-based treatment for AN. While both the health (14-17,45-47) and financial costs (40-43) of AN are extremely high, if AN can be effectively treated early on long-term costs can potentially be minimized.

7.1 Body Composition

Short-term recovery from AN is associated with increased gain of fat mass (FM) relative to lean body mass (LBM) and higher central adiposity than those

without a history of an ED (2-4,71,90-92). Similarly, persons that experienced undernutrition in early childhood show increased gain of FM relative to LBM (86,196) and increased central adiposity compared to peers that did not experience undernutrition (5,6,9,178,305-307). Therefore, it seems likely that RAN individuals could be at increased risk for large gain of central fat mass and associated chronic diseases.

Data from our study suggests that long-term recovery from AN is not associated with a higher central adiposity compared to control (C) participants. Briefly, RAN and C participants had comparable FM, LBM, truncal fat mass (TrFM), and extremity fat. Even when adjusting for total FM, TrFM did not differ between groups. Hence, in individuals with a history of AN, the time following undernutrition has important implications for body composition.

7.1.1 Future Areas of Research

While we did not find any differences between groups in body composition or body fat distribution, it is possible that RAN participants have more visceral or intramuscular adipose tissue than C participants. In fact, it has been shown that in short-term recovery from AN, both visceral adipose tissue and intramuscular adipose tissue are higher in persons with a history of AN compared to C participants (4). Therefore, additional studies should measure more explicit components of body fat distribution, such as visceral adipose tissue and intramuscular adipose tissue, in RAN individuals as it could have important health implications.

7.2 Cortisol

Adults in short-term recovery following AN, stunted children and some data on persons born SGA have associated increased cortisol levels with undernutrition (2,4,8,124,141-146,152,154,160-163). Yet, little information exists on cortisol levels or cortisol response to stress in long-term recovery following AN.

We found that undernutrition occurring after puberty has different long-term outcomes than undernutrition occurring in utero or during early childhood. Further, the time following starvation is important for cortisol levels long-term. Unlike the other the groups, RAN participants had cortisol levels comparable to C participants at each of the 5 baseline time points and 7 Trier Social Stress Test (TSST) time points. Area under the curve for cortisol during the TSST also did not differ between groups. This indicates that the response to the stress of the TSST was similar between RAN and C participants. While the TSST significantly increased cortisol levels in both RAN and C participants, the magnitude of the increase did not differ between groups. Based on these findings, it does not seem that RAN individuals would be at increased risk for central fat gain.

7.2.1 Future Areas of Research

While it seems that higher degrees of perfectionism and body satisfaction found in our RAN participants may have been associated with increased cortisol levels, we found similar cortisol levels, an observation that should be further explored. It is possible that the extreme stress of starvation re-sets the stress threshold at which cortisol is released. It is also possible that though still

experiencing more perfectionism and body dissatisfaction than C participants, the RAN participants could have experienced a decrease in perfectionism and body dissatisfaction from the time at which the participant had AN. If this is the case, the body may no longer respond to these as being stressful and therefore cortisol levels may not be higher than C participants. This needs to be further studied and mechanisms elucidated.

Our study only evaluated psychological stress in RAN individuals. Data from other studies indicate that physical stress, like exercise, is also associated with increased cortisol levels in persons with EDs (314). Further studies are needed to determine if under physical stress, cortisol secretion is comparable between RAN and C participants. It is possible that since RAN individuals have undergone such severe physical stress the body may be more responsive to physical stress as compared to psychological stress.

7.3 Resting Energy Expenditure

Resting energy expenditure (REE) has also been shown to be lower in persons with AN than persons without a history of an ED and then predicted by prediction equations (179-184). In short-term recovery from AN, REE increases from AN (93,180,186-191), but may not reach that of individuals without a history of an ED (183,184,320).

Our data suggests that in long-term recovery following AN, REE does not differ between RAN and C participants; similarities remain when adjusting for LBM. Therefore, akin to body composition and cortisol levels, the time following undernutrition may have important implications on REE. Further, REE

comparable to controls indicates that RAN individuals are not at increased risk for gain of weight or FM, consistent with our body composition results.

7.3.1 Future Areas of Research

Future research could explore the relationship between REE and total energy expenditure in individuals in long-term recovery from AN. While REE did not differ between RAN and C participants, it is possible that total energy expenditure differs between groups and could have implications on adequate caloric intake for this population.

7.4 Substrate Oxidation

Impaired fat oxidation has been shown in stunted children, overweight and obese individuals who have lost and regained weight and people in re-feeding from AN (9,201,204). However, shortly following re-feeding, RQ values did not differ between AN and C subjects (183,184) and 6 months following weight regain from AN fat utilization was increased (188). Still, little information is available regarding substrate oxidation following re-feeding and substrate oxidation in an AN sample, controlling for diet.

We found that RAN women have a low RQ and higher fat oxidation compared to C women. Thus, it appears that both undernutrition following early childhood and the time following undernutrition result in changes in substrate oxidation. A lower RQ in RAN compared to C women should not predispose this group to increased gain of FM and chronic diseases.

7.4.1 Future Areas of Research

Further research is needed to elucidate the precise mechanisms that are responsible for the lower RQ found in RAN compared to C participants. Adiponectin or Peptide YY are two hormones that may be related to the low RQ and increased fat metabolism and should warrant further research. Also, as we did not measure postprandial substrate oxidation, differences may also exist and should be further studied.

Additional research is also needed to elucidate the mechanism responsible for the relationship between substrate oxidation and body composition in long-term recovery following AN. Based on our data is seems as though RAN participants could have had lower FM or TrFM than C participants, but this was not found. Future studies should explore the health implications of RQ lower than that of C participants following undernutrition.

7.5 Perfectionism

Perfectionism is common in persons with AN (209-213) and persists into recovery from AN (65,170,213,215,216,221). Furthermore, perfectionism has been associated with a higher cortisol stress response to the TSST (222). Therefore perfectionism could potentially contribute to higher cortisol levels in RAN individuals.

Data from our study indicated that overall perfectionism is not higher, but nondisclosure of imperfection (hiding imperfection from others) is significantly higher in RAN compared to C individuals. Questions asked during the TSST likely asked persons to reveal imperfections and participants were asked to

perform tasks that were designed to be nearly impossible in front of a panel of strangers. Based on earlier research, it seems that cortisol levels could have been higher in the RAN women. However, RAN participants did not have increased cortisol levels or heart rate compared to C participants.

7.5.1 Future Areas of Research

Similar research should be repeated in a group of persons in long-term recovery from AN who score higher than control participants on overall perfectionism as well as individual components of perfectionism. It is possible that increased cortisol is only seen when overall perfectionism is high and not one specific component of perfectionism.

7.6 Body Satisfaction

Not only do persons with EDs show higher negative emotion surrounding body image (240), but persons who are more concerned about their body image have higher afternoon cortisol levels (241). Therefore, body satisfaction could contribute to increased cortisol levels in RAN women in a baseline state or stressed state.

Data from this study indicate a trend for RAN women to exhibit higher levels of body dissatisfaction than C women. Furthermore, while a score of 90 on the Body Shape Questionnaire (BSQ) is indicative of normative body dissatisfaction (237,239) our RAN volunteers scored 100 indicating greater than normative body dissatisfaction. Despite scoring higher than C participants and having higher than normative body dissatisfaction, no differences in cortisol or cortisol stress response were found. It is possible that despite the higher body

dissatisfaction the similarities to C participants in responses to question 15, indicative of body checking, or question 30, indicative of body avoidance, are important in mitigating a cortisol response.

7.6.1 Future Areas of Research

In this study there was only a trend for higher body dissatisfaction in RAN women. It is possible that the difference in body dissatisfaction between our RAN and C individuals were not robust enough to produce differences in cortisol levels. Therefore similar research should be conducted on RAN individuals who differ greatly from normative body dissatisfaction and persons without a history of an eating disorder. Further research should also explore the relationship between cortisol levels and answers to questions 15 and 30 on the BSQ.

7.7 Other Areas of Future Research

7.7.1 Depression and Anxiety Disorders

It would be important to look at several psychological factors that may be associated with increased cortisol levels in some RAN persons. Our study excluded potential participants with depression and anxiety disorders. Co-morbidity of psychological disorders including depression and anxiety disorders are somewhat prevalent in persons with EDs overall, AN not being an exception (16,39,64,65,340-346). It has been shown that even in recovery from AN, co-morbid disorders may persist (64,65). Depression and anxiety disorders are associated with alterations in cortisol levels compared to persons without a history of psychological disorders (347-356). Therefore, it is possible that RAN individuals with co-morbid, persisting, psychological disorders may have

metabolic alterations that we did not see in our RAN participants. Thus, future research should explore the relationship between RAN participants with and without a co-morbid psychological disorder, a group with the psychological disorder and no history of an ED, and a control group with no history of the psychological disorder and no history of an ED to determine if differences in cortisol exist. If differences in cortisol are found, these differences could impact both body composition and substrate oxidation.

7.7.2 Treatment of AN

The speed at which re-feeding occurs could impact body composition (2,4) and cortisol. In addition, the diet fed during and following re-feeding would impact substrate oxidation. Future research should explore if long-term differences in body composition, cortisol levels, and substrate oxidation are associated with different re-feeding speeds or dietary compositions. It is possible that slower weight gain could have an impact on body fat distribution, short or long-term. Further, it is possible that more rapid weight regain could result in higher anxiety, but for a shorter time period. This could have implications for the Hypothalamic-Pituitary-Adrenal axis and cortisol secretion. In addition, the composition of the diet could impact both cortisol levels (depending on the stress associated with the food items being fed) and with substrate oxidation. This could then impact body composition. Therefore exploring the relationship between these factors could help in the development of more effective treatment.

Future studies on RAN individuals should ask participants about what sort of treatment the person was given. As effective, evidence-based treatment for

AN is lacking, exploring which treatments worked for RAN individuals could help elucidate which treatments might be worth further developing. Perhaps evaluating treatment in RAN individuals could help improve effective treatment of this disorder.

7.7.3 Longitudinal Studies Are Needed

Research presented here is among the first body of research to study body composition, cortisol levels, energy metabolism, perfectionism, and body satisfaction in women in long-term recovery from AN in a single study. Furthermore, this study is unique in that it used a group of women who had been recovering from AN for 2 or more years, something that has not been done by other studies. It seems that the time following undernutrition may have an important impact on overall body composition, cortisol, and energy metabolism. Our findings suggest that in long-term recovery from AN, the women studied are more similar to women without a history of an eating disorder than women in short-term recovery from AN. Also, there are less differences between RAN and C participants in perfectionism and body satisfaction than between persons with AN and persons without a history of an ED. However, we do not have data from our RAN individuals during or shortly following AN. Therefore, we were unable to assess how body composition, body fat distribution, cortisol levels, REE, substrate oxidation, perfectionism and body satisfaction changed over time. A longitudinal study allowing researchers to examine these changes over time would greatly benefit the research community.

7.8 Treatment implications

Overall, this research has important treatment implications. In the treatment of AN, psychologists and other clinicians can use information learned here to address clients concerns regarding the gain of central adiposity. This could help to improve treatment and recovery rates for persons with AN. Knowing the long-term risks associated with AN can help medical professional best treat these individuals. For example, knowing this group is not likely at increased risk for gain of central fat mass or associated chronic disease can help focus office visits to persistent problems, like osteoporosis or elevated cholesterol, and maximize benefits to the client.

7.9 SUMMARY

In summary, the long-term prognosis in those able to recover from AN is exceptionally promising. Following successful re-feeding, RAN individuals have body composition, body fat distribution, and cortisol levels at baseline and under stress similar to persons without a history of an ED. Cortisol reactivity to stress is also similar between RAN and C women. Furthermore, RQ is decreased and fat oxidation is increased. Therefore, RAN individuals differ from persons that experienced starvation in early childhood and in short-term recovery following AN. Furthermore, based on data presented in this dissertation, RAN individuals are not at increased risk for gain of fat mass, gain of central fat or associated chronic diseases.

8 APPENDIX

8.1 Appendix A: Selected Data

Subject Number	Height (cm)	Weight (kg)	BMI	Age (yrs)	Group
7	173	64.1	21.42	28.5	C
8	173	68.7	22.95	27.4	C
9	166	67.5	24.50	24.1	C
12	172	60.7	20.52	19.8	C
13	159	50.9	20.14	19.6	C
19	158	53.6	21.47	22.3	C
22	160	62.9	24.73	28.5	C
24	168	68.4	24.38	22.5	C
25	173	69.5	23.28	20.5	C
26	166	61.6	22.46	22.3	C
29	165	67.9	24.97	23.4	C
34	176	80.5	25.99	21.6	C
35	178	67.2	21.21	21.3	C
37	164	76.2	28.51	35.8	C
39	157	45.9	18.621	31.1	C
40	167	55.7	20.00	23.5	C
43	162	64.3	24.47	20.7	C
44	164	57.1	21.26	19.3	C
1	170	68.6	23.75	24.4	RAN
3	176	72.7	23.47	21.8	RAN
4	165	69.1	25.38	21.3	RAN
6	167	69.9	25.06	29.1	RAN
11	164	59.0	22.07	18.9	RAN
30	173	67.7	22.75	19.6	RAN
32	175	65.5	21.39	18.6	RAN
33	161	48.1	18.56	20.1	RAN
38	170	58.2	20.26	25.2	RAN
41	168	63.9	22.78	25.1	RAN
45	155	47.1	19.61	34.2	RAN
55	167	52.1	18.61	23.6	RAN
57	168	65.5	23.21	20.1	RAN
58	164	54.4	20.13	33.5	RAN
61	151	59.3	26.00	19.6	RAN
63	164	54.6	20.40	21.3	RAN

Subject Number	Lowest Adult Weight (kg)	Lowest Adult Body Mass Index	Recovery Months	Body Shape Questionnaire-34	Perfectionistic Self-Presentation Scale
7	61.36	20.50	.	59	108
8	61.36	20.50	.	137	81
9	59.09	21.44	.	47	82
12	61.36	20.74	.	54	71
13	46.82	18.52	.	54	84
19	50.91	20.39	.	72	70
22	56.82	22.33	.	74	84
24	61.36	21.87	.	107	105
25	59.09	19.78	.	123	133
26	54.55	19.89	.	85	108
29	61.36	22.67	.	93	133
34	68.64	22.16	.	82	101
35	65.00	20.52	.	54	104
37	54.55	20.40	.	91	94
39	44.55	18.07	.	81	113
40	50.00	17.95	.	56	73
43	58.64	22.32	.	68	119
44	50.00	18.60	.	129	111
1	43.64	15.10	87	90	142
3	52.27	16.88	33	120	154
4	31.82	11.69	75	99	99
6	40.91	14.67	75	51	58
11	40.46	15.13	27	107	84
30	61.36	20.62	49	81	66
32	47.73	15.58	24	95	117
33	40.91	15.78	75	115	130
38	50.00	17.40	120	46	115
41	54.55	19.44	85	87	107
45	32.27	13.43	144	94	116
55	48.64	17.38	72	76	92
57	47.27	16.75	36	126	89
58	44.55	16.48	228	130	93
61	50.00	21.93	49	188	54
63	43.64	16.30	54	108	146

Subject Number	VCO_2	VO_2	Respiratory Quotient	Food Quotient	Resting Energy Expenditure
7	.167	.191	.87	.86	1350
8	.190	.210	.91	.86	1494
9	.197	.224	.88	.86	1528
12	.177	.194	.91	.88	1380
13	.134	.152	.88	.85	1078
19	.167	.161	1.04	.85	1177
22	.176	.191	.92	.86	1363
24	.183	.200	.91	.85	1428
25	.178	.198	.90	.86	1405
26	.166	.197	.84	.86	1383
29	.165	.184	.90	.85	1308
34	.163	.186	.88	.85	1317
35	.197	.214	.92	.85	1526
37	.169	.196	.86	.85	1379
39	.140	.164	.86	.84	1153
40	.178	.184	.97	.85	1325
43	.148	.164	.90	.83	1164
44	.145	.181	.80	.86	1257
1	.171	.191	.89	.86	1358
3	.169	.190	.89	.86	1346
4	.175	.216	.81	.86	1502
6	.188	.203	.92	.86	1454
11	.139	.183	.76	.87	1260
30	.160	.198	.81	.86	1379
32	.174	.196	.89	.86	1389
33	.145	.173	.84	.87	1209
38	.158	.191	.83	.86	1334
41	.162	.197	.82	.86	1374
45	.112	.138	.81	.85	961
55	.136	.163	.84	.85	1140
57	.145	.167	.87	.84	1179
58	.132	.154	.86	.87	1084
61	.117	.132	.89	.91	961
63	.156	.202	.78	.86	1393

Subject Number	Percent Trunk Tissue Fat	Percent Trunk Region Fat	Trunk Tissue (g)	Trunk Fat (g)	Trunk Lean (g)
7	36.0	34.8	26552	9552	17000
8	31.7	30.7	32262	10240	22022
9	31.6	30.6	29955	9456	20499
12	26.7	25.9	26516	7085	19430
13	22.8	22.3	20640	4716	15924
19	21.7	21.1	23835	5176	18659
22	28.0	27.0	28641	8012	20628
24	36.1	34.8	28389	10258	18131
25	35.3	34.1	29636	10452	19184
26	32.0	31.1	25270	8083	17187
29	39.2	38.1	30427	11918	18510
34	17.2	16.9	30049	5174	24875
35	22.3	21.5	32062	7143	24919
37	43.9	43.1	33482	14714	18768
39	17.5	17.0	20458	3576	16882
40	29.0	28.1	22755	6604	16151
43	42.0	40.9	29015	12199	16816
44	36.2	35.1	25596	9277	16319
1	28.1	27.6	26439	7441	18998
3	45.3	43.7	33146	14999	18147
4	28.3	27.5	26875	7614	19262
6	33.2	32.3	29136	9682	19454
11	28.5	27.9	25515	7276	18239
30	33.1	32.2	29488	9763	19725
32	19.6	19.0	26377	5169	21208
33	24.1	23.4	22158	5336	16823
38	25.6	24.9	24369	6237	18132
41	8.7	8.5	28261	2447	25814
45	21.6	21.0	20809	4500	16309
55	16.7	16.2	21553	3592	17961
57	34.4	33.5	28040	9655	18386
58	25.1	24.4	23114	5802	17321
61	46.7	45.5	27669	12910	14759
63	15.5	15.1	21088	3264	17825

Subject Number	Percent Total Tissue Fat	Percent Total Region Fat	Total Tissue (g)	Total Fat (g)	Total Lean (g)
7	33.7	32.2	61076	20588	40488
8	32.1	30.7	66397	21335	45062
9	35.0	33.6	65345	22871	42474
12	28.8	27.5	56966	16385	40582
13	26.1	25.1	48057	12566	35491
19	25.0	23.9	51093	12793	38300
22	27.9	26.6	59853	16689	43164
24	36.8	35.0	64700	23785	40915
25	33.8	32.4	66196	22399	43798
26	37.4	35.8	57679	21549	36130
29	40.8	39.1	64405	26253	38152
34	24.0	23.1	72644	17415	55229
35	23.0	21.9	65705	15107	50599
37	43.4	42.2	72938	31677	41261
39	22.4	21.4	43655	9795	33859
40	32.0	30.6	52496	16809	35687
43	39.4	37.8	61543	24250	37293
44	35.8	34.3	53474	19159	34315
1	31.5	30.5	66012	20786	45226
3	41.7	39.8	69504	28966	40538
4	30.5	29.2	63917	19469	44448
6	32.0	30.7	66198	21206	44992
11	30.8	29.7	57058	17583	39476
30	31.1	29.8	64158	19936	44222
32	25.7	24.7	61717	15875	45842
33	25.6	24.4	45620	11661	33959
38	29.7	28.5	54814	16286	38528
41	15.2	14.6	63031	9562	53470
45	25.2	24.1	43954	11090	32864
55	20.5	19.6	49122	10057	39065
57	38.0	36.6	60844	23140	37704
58	31.8	30.5	52385	16681	35704
61	42.8	41.3	55661	24265	32395
63	24.3	23.4	51003	12396	38607

Subject Number	Bone Mineral Density (g/cm^2)	Bone Mineral Content (g)	Spine (g/cm^2)	Pelvis (g/cm^2)
7	1.202	2831	1.015	1.130
8	1.349	3143	1.237	1.255
9	1.194	2722	1.019	1.219
12	1.139	2614	1.141	1.148
13	1.139	2034	.899	.965
19	1.190	2393	.946	1.128
22	1.311	2812	1.113	1.350
24	1.294	3288	1.267	1.205
25	1.252	3030	1.022	1.257
26	1.138	2435	1.023	1.060
29	1.170	2695	.926	1.105
34	1.185	2699	.847	1.076
35	1.302	3128	1.087	1.304
37	1.080	2153	.850	1.089
39	1.142	2160	.807	1.019
40	1.083	2378	.946	1.099
43	1.261	2608	1.023	1.378
44	1.064	2380	.980	1.119
1	1.035	2075	.775	1.071
3	1.213	3205	1.078	1.205
4	1.211	2744	1.052	1.184
6	1.296	2950	1.067	1.354
11	1.152	2134	.976	1.071
30	1.237	2818	1.144	1.234
32	1.163	2671	.944	1.196
33	1.157	2151	.979	1.165
38	1.079	2315	.873	1.094
41	1.128	2494	.843	1.078
45	1.160	2075	1.032	1.125
55	1.137	2244	.949	1.199
57	1.094	2376	.895	1.091
58	1.153	2304	.880	1.118
61	1.041	2083	.834	.967
63	1.046	1983	.766	.928

Subject Number	Cortisol Awake (nM)	Cortisol Awake +15 (nM)	Cortisol Awake +30 (nM)	Cortisol 3pm (nM)	Cortisol Bed (nM)
7	7.70	11.81	9.77	0.99	0.94
8	15.32	15.38	18.80	0.90	1.97
9	12.91	24.30	40.39	5.71	5.87
12	12.88	26.46	37.08	6.73	11.86
13	34.99	38.13	61.33	0.08	4.56
19	9.79	15.70	22.94	0.34	1.14
22	8.54	20.24	32.89	4.20	1.81
24	.	.	.	.	.
25	13.28	39.28	56.71	1.26	0.30
26	57.30	67.32	48.31	76.10	90.02
29	8.24	13.64	14.89	0.60	0.86
34	.	.	.	.	.
35	.	.	.	.	.
37	3.85	5.57	7.87	0.80	1.26
39	.	.	.	.	.
40	2.10	22.91	62.52	0.22	0.25
43	.	.	.	.	.
44	.	.	.	.	.
1	16.47	18.03	23.42	4.44	1.12
3	5.74	4.18	10.25	3.63	1.19
4	3.12	4.55	11.55	1.34	0.52
6	29.80	77.65	70.02	3.59	2.44
11	5.41	7.03	14.15	1.54	0.85
30	.	.	.	.	.
32	22.00	43.64	34.13	0.817	1.76
33	22.09	16.21	19.09	2.55	7.44
38	3.06	2.81	6.17	1.85	0.30
41	.	8.16	17.73	1.06	0.29
45	.	.	.	.	.
55	34.04	69.07	13.24	47.99	21.69
57	1.05	18.54	53.40	25.73	3.25
58	8.97	65.01	78.86	7.28	0.70
61	1.28	6.50	13.86	11.87	0.61
63	10.69	35.53	45.72	6.99	.

Subject Number	Cortisol Arrival (nM)	Cortisol After Equipment Placement (µM)	Cortisol Finish TSST (nM)	Cortisol Finish TSST +10 (nM)	Cortisol Finish TSST +20 (nM)
7	14.492	11.954	21.788	7.634	6.24
8	2.225	2.521	7.052	6.769	2.15
9	3.891	9.114	6.778	7.876	3.79
12	1.432	2.443	12.860	6.996	4.69
13	4.245	7.984	37.093	60.396	60.95
19	1.114	.367	2.485	4.378	5.69
22	3.344	1.714	9.451	2.551	8.47
24	.	.	.	.	.
25	16.715	19.830	26.708	33.561	27.26
26	160.751	145.576	100.508	135.143	75.06
29	1.014	.982	8.236	20.385	16.90
34	.	.	.	.	.
35	.	.	.	.	.
37	1.083	2.419	15.053	30.503	20.17
39	.	.	.	.	.
40	3.554	1.489	9.440	58.251	56.16
43	.	.	.	.	.
44	.	.	.	.	.
1	1.401	3.063	4.905	6.354	4.98
3	.470	3.228	3.677	3.272	4.23
4	1.596	4.275	4.878	5.294	2.75
6	11.439	7.781	18.385	53.125	38.01
11	2.857	5.603	13.863	7.646	5.66
30	.	.	.	.	.
32	6.068	7.214	31.517	29.774	22.00
33	11.141	12.251	72.708	94.131	94.13
38	.716	1.765	26.627	2.030	1.87
41	5.055	5.911	5.574	4.107	3.26
45	.	.	.	.	.
55	1.296	16.461	23.563	24.222	11.32
57	16.189	17.369	19.138	16.466	9.97
58	4.598	4.612	24.748	31.146	16.72
61	6.842	16.346	22.571	13.077	12.25
63	7.781	5.089	25.544	19.704	14.62

Subject Number	Cortisol Finish TSST +30 (nM)	Cortisol Finish TSST +45 (nM)
7	5.583	2.651
8	2.923	1.796
9	3.708	3.113
12	2.522	4.203
13	25.362	32.116
19	3.163	1.435
22	5.157	1.523
24	.	.
25	28.289	9.356
26	153.609	81.750
29	16.331	22.471
34	.	.
35	.	.
37	16.962	9.336
39	.	.
40	45.214	54.543
43	.	.
44	.	.
1	2.837	3.268
3	.769	.656
4	2.230	1.901
6	24.581	13.672
11	5.743	4.322
30	.	.
32	11.607	9.587
33	63.127	101.268
38	1.339	1.262
41	2.753	3.026
45	.	.
55	15.434	7.959
57	4.336	6.678
58	4.500	1.587
61	6.393	6.240
63	7.650	5.126

8.2 Appendix B: Questionnaires

8.2.1 Screening Questionnaire

Volunteer Number: _____
Male/Female: _____
Age:_____
Ethnicity:_____
Birth weight:_____
Current Medications:

_____ _____ _____
_____ _____ _____
_____ _____ _____

List Chronic Conditions asthma, diabetes…)_____
_____
I am currently battling an eating disorder (y/n)

I have suffered from Anorexia Nervosa in the past (y/n)
If Yes: I have been in recovery for _____ Years.

I have suffered from an Eating Disorder other than Anorexia Nervosa (y/n)
If Yes: I have been in recovery _____ Years.

Have you ever experienced Amenorrhea (loss of 3 consecutive menstrual periods?) (y/n)
If yes for how long_____
If yes how long of you been regularly menstruating?_____

In the past, I was afraid of gaining weight even though I was underweight (y/n)
If yes, how long ago?_____

Currently, I am afraid of gaining weight even though I am underweight (y/n)

In the past, my body weight greatly influenced how I felt about myself (y/n)
If yes, how long ago?_____

Currently, my body weight greatly influenced how I felt about myself (y/n)
I currently binge and/or purge (y/n)
If yes, how often?_____

My lowest adult weight was_____.
My highest adult weight is_____.
My weight has been stable $\pm$ 5 lbs for _____ Months.
Hours of exercise per week_____.

Are you pregnant or do you plan to become pregnant in the near future (y/n)
I have or currently smoke (y/n)
If yes how many cigarettes per day_____
I have used/currently use drugs (y/n)
Do you drink alcohol (y/n)
If yes how many drinks per week_____
I am a vegetarian or would prefer to eat vegetarian meals if selected for the study (y/n)
Are you currently on a diet (y/n)
If Yes please explain:_____
Food Allergies_____
Other Allergies_____

Do Not Complete
Height_____
Weight_____
BMI_____

8.2.2 Perfectionistic Self-Presentation Scale

PSPS

Listed below are a group of statements. Please rate your agreement with each of the statements using the following scale. If you strongly agree, circle 7; if you disagree, circle 1; if you feel somewhere in between, circle any one of the numbers between 1 and 7.
If you feel neutral or undecided the midpoint is 4.

1. It is okay to show others that I am not perfect……
   1 2 3 4 5 6 7

2. I judge myself based on the mistakes I make in front of other people……
   1 2 3 4 5 6 7

3. I will do almost anything to cover up a mistake……..
   1 2 3 4 5 6 7

4. Errors are much worse if they are made in public rather than in private……
   1 2 3 4 5 6 7

5. I try always to present a picture of perfection…….
   1 2 3 4 5 6 7

6. It would be awful if I made a fool of myself in front of others……..
   1 2 3 4 5 6 7

7. If I seem perfect, others will see me more positively…… .
   1 2 3 4 5 6 7

8. I brood over mistakes that I have made in front of others……
   1 2 3 4 5 6 7

9. I never let others know how hard I work on things…….
   1 2 3 4 5 6 7

10. I would like to appear more competent than I really am…….
    1 2 3 4 5 6 7

11. It doesn't matter if there is a flaw in my looks……..
    1 2 3 4 5 6 7

12. I do not want people to see me do something unless I am very good at it….
    1 2 3 4 5 6 7

13. I should always keep my problems to myself…….
    1 2 3 4 5 6 7

14. I should solve my own problems rather than admit them to others……..
    1 2 3 4 5 6 7

15. I must appear to be in control of my actions at all times…….
    1 2 3 4 5 6 7

16. It is okay to admit mistakes to others……
1 2 3 4 5 6 7

17. It is important to act perfectly in social situations……..
1 2 3 4 5 6 7

18. I don't really care about being perfectly groomed……..
1 2 3 4 5 6 7

19. Admitting failure to others is the worst possible thing……..
1 2 3 4 5 6 7

20. I hate to make errors in public……
1 2 3 4 5 6 7

21. I try to keep my faults to myself……..
1 2 3 4 5 6 7

22. I do not care about making mistakes in public……
1 2 3 4 5 6 7

23. I need to be seen as perfectly capable in everything I do…….
1 2 3 4 5 6 7

24. Failing at something is awful if other people know about it…….……
1 2 3 4 5 6 7

25. It is very important that I always appear to be "on top of things"……
1 2 3 4 5 6 7

26. I must always appear to be perfect……
1 2 3 4 5 6 7

27. I strive to look perfect to others……..
1 2 3 4 5 6 7

* Hewitt PL, Flett GL, Ediger E. Perfectionism traits and perfectionistic self-presentation in eating disorder attitudes, characteristics, and symptoms. Int J Eat Disord 1995;18:317-26.
**Scale and Scoring provided by the Hewitt Lab.

8.2.3 Body Shape Questionnaire-34

BSQ-34

We should like to know how you have been feeling about your appearance over the PAST FOUR WEEKS. Please read each question and circle the appropriate number to the right. Please answer all the questions.

OVER THE PAST FOUR WEEKS:

		Never	Rarely	Sometimes	Often	Very often	Always
1.	Has feeling bored made you brood about your shape?.....	1	2	3	4	5	6
2.	Have you been so worried about your shape that you have been feeling you ought to diet?.....	1	2	3	4	5	6
3.	Have you thought that your thighs, hips or bottom are too large for the rest of you?.....	1	2	3	4	5	6
4.	Have you been afraid that you might become fat (or fatter)?.....	1	2	3	4	5	6
5.	Have you worried about your flesh being not firm enough?.....	1	2	3	4	5	6
6.	Has feeling full (e.g. after eating a large meal) made you feel fat?.....	1	2	3	4	5	6
7.	Have you felt so bad about your shape that you have cried?.....	1	2	3	4	5	6
8.	Have you avoided running because your flesh might wobble?.....	1	2	3	4	5	6
9.	Has being with thin women made you feel self-conscious about your shape?.....	1	2	3	4	5	6
10.	Have you worried about your thighs spreading out when sitting down?	1	2	3	4	5	6
11.	Has eating even a small amount of food made you feel fat?.....	1	2	3	4	5	6
12.	Have you noticed the shape of other women and felt that your own shape compared unfavourably?.....	1	2	3	4	5	6
13.	Has thinking about your shape interfered with your ability to concentrate (e.g. while watching television, reading, listening to conversations)?.....	1	2	3	4	5	6
14.	Has being naked, such as when taking a bath, made you feel fat?.....	1	2	3	4	5	6
15.	Have you avoided wearing clothes which make you particularly aware of the shape of your body?.....	1	2	3	4	5	6
16.	Have you imagined cutting off fleshy areas of your body?.....	1	2	3	4	5	6

		Never	Rarely	Sometimes	Often	Very often	Always
17.	Has eating sweets, cakes, or other high calorie food made you feel fat?	1	2	3	4	5	6
18.	Have you not gone out to social occasions (e.g. parties) because you have felt bad about your shape?.....	1	2	3	4	5	6
19.	Have you felt excessively large and rounded?.....	1	2	3	4	5	6
20.	Have you felt ashamed of your body?.....	1	2	3	4	5	6
21.	Has worry about your shape made you diet?.....	1	2	3	4	5	6
22.	Have you felt happiest about your shape when your stomach has been empty (e.g. in the morning)?.....	1	2	3	4	5	6
23.	Have you thought that you are in the shape you are because you lack self-control?.....	1	2	3	4	5	6
24.	Have you worried about other people seeing rolls of fat around your waist or stomach?.....	1	2	3	4	5	6
25.	Have you felt that it is not fair that other women are thinner than you?.	1	2	3	4	5	6
26.	Have you vomited in order to feel thinner?.....	1	2	3	4	5	6
27.	When in company have your worried about taking up too much room (e.g. sitting on a sofa, or a bus seat)?.....	1	2	3	4	5	6
28.	Have you worried about your flesh being dimply?.....	1	2	3	4	5	6
29.	Has seeing your reflection (e.g. in a mirror or shop window) made you feel bad about your shape?.....	1	2	3	4	5	6
30.	Have you pinched areas of your body to see how much fat there is?.....	1	2	3	4	5	6
31.	Have you avoided situations where people could see your body (e.g. communal changing rooms or swimming baths)?.....	1	2	3	4	5	6
32.	Have you taken laxatives in order to feel thinner?.....	1	2	3	4	5	6
33.	Have you been particularly self-conscious about your shape when in the company of other people?.....	1	2	3	4	5	6
34.	Has worry about your shape made you feel you ought to exercise?.....	1	2	3	4	5	6

*Cooper PJ, Taylor, M. J., Cooper, Z. and Fairburn, C. G. The development and validation of the Body Shape Questionnaire. International Journal of Eating Disorders 1987;6:485-494.

8.2.4 Trier Social Stress Test Script

Trier Social Stress Test Script-Adapted

Primary Investigator Script

"**Your** task in this experiment is to present an introductory talk in front of this committee. Please imagine that you had applied for a **job** and have been invited for an **interview**. In contrast to a real interview, however, you are supposed to give a talk, in which you are to convince the committee in **five** minutes why you think that you would be the best candidate for this position. Please note that you will be recorded by a **camera** (pause, point to the camera) and a **microphone** (pause, point to the microphone) for later voice and behavioral analysis. The members of the committee are trained in behavioral analysis and will take notes during your talk. You should try to leave the best possible impression, and assume the role of the applicant for the duration of the talk as best as you can. The committee will reserve the right to ask follow-up questions in case of uncertainties to receive all necessary information from you. Following your talk you will then be given a second task **by the committee** which will **only** be explained to you by the committee. Questions considering the experiment are **not** allowed to be asked during the task, do you have any questions now? … I will now leave the laboratory and come back after the test is finished. You may now take some notes for few minutes which you must not use during your talk. However, during the talk it is most importance that you do not move too much. **The committee will tell you when to start**. Good luck."

Panel Script

The Trier Social Stress Test (TSST) Script

DO NOT SAY BOLDED

3 minutes

- Three minutes preparation time (*Scribble on paper)*

After 3 minutes

- "We are now ready to begin please step to the microphone" (*camera and recorder are switched on, notes taken from subjects).*

5 minutes

- *Keep straight face, no nodding or emotion, scribble (or draw, whatever but make it look scary DO NOT TAKE ACTUAL NOTES ON THE PERSON)*
- The first minute(s) should nevertheless be kept in silence.
- Only after a pause of more than twenty seconds prior to the end of the five-minute period, questions are asked,
    - "You still have time, please continue...". **USE THIS FIRST**
    - "What qualifies you in particular for this position?"
    - Why do you think that you are the best applicant for this position?
    - Do you have any bad qualities? (After stating few, ask about other bad qualities, everyone has learned one or two "good bad qualities". Or state that "those are usually not bad qualities, could you name others?)
    - Why is that a good quality?
    - What qualities **other** people respect about you?

- Which leader-qualities do you have?

If speaking fluently at 3 minutes, interrupt with irrelevant question **MAKE SURE TO INTERJECT AT LEAST 1 Time**

- **DO NOT USE THE FOLLOWING QUESTIONS IF A PAUSE HAS OCCURED**
  - What do you think about no smoking policy in restaurants and bars
  - What do you think about BMI on children's report cards?
  - What do you think about Trans fat bans?
  - Who is your favorite president and why?

NOTE THE NUMBER OF PROMPTS AND THE TIME OF THE FIRST PAUSE/INTERRUPTION

Some S's use their school-knowledge to distract from their own person. In that case the chair should certainly intervene, for example by saying: "These experiences can be drawn from your CV, we would in this interview be more interested about your personality."

After the five-minute-period (8 minutes into the test)

"Thank you that is enough"

- "We now want you to solve a calculation task. Please count aloud backwards from 2023 to zero in 17-step sequences. Please calculate as quickly and correctly as possible. Should you miscalculate, we will point out your mistake and you have to start all over again. Do you have any questions? Please begin, then."
- If mistake is made "Wrong Please start from 2023." until the end of the test period. (*Note the number of errors and the number that the subject eventually reached as a performance measure*.)

After 5 Minutes- (13 minutes into the test)

- At the end of the test period the chair should thank the S for his/her participation I will come and collect cortisol, BP and Heart Rate
- Come to Indirect Calorimeter room after about 10 minutes

*http://www.macses.ucsf.edu/Research/Allostatic/notebook/challenge.html#General

Developed by Clemens Kirschbaum, Ph.D.; Institut für Physiologische Psychologie II; Universitätsstrasse 1; D-40225 Duesseldorf, Germany.- adapted.

8.3 Appendix C: Additional Results

8.3.1 Resting Energy Expenditure and Bone Mineral Content

Objective: To determine if lower bone mass is correlated with lower resting energy expenditure (REE) in persons with a history of Anorexia Nervosa and in Recovery for 2 or more years (RAN) compared to control (C) participants.

REE (kcal/day) / bone mineral content (g) did not differ between RAN and C participants (0.53 ± 0.07 v. 0.51 ± 0.05, respectively; (p = 0.363). **Table 8-1a** shows the relationship between REE and bone mineral content in RAN and C women. While group was not significantly associated with REE, bone mineral content was significantly associated with REE (p = 0.000). Persons with higher bone mass had higher REE. It should be noted that RAN participants had a trend for lower bone mineral content (P = 0.069). When including fat mass and lean body mass in the model, REE was more strongly associated with lean body mass than bone mineral content **Table 8-1b**, as expected.

Table 8-1a Resting Energy Expenditure and Bone Mineral Content in Women Recovered from Anorexia Nervosa ≥ 2 Years (RAN) and Control (C) Women

Resting Energy Expenditure (R^2 = 0.377)

VARIABLE	Coefficient	Standard Error	p-value
Constant	689.305	156.051	0.000
Group*	-14.13	43.293	0.746
Bone Mineral Content (g)	0.247	0.058	0.000[1]

***C = 0; RAN = 1**

[1]Partial Eta^2 = 0.377

Table 8-1b Resting Energy Expenditure and Body Composition in Women Recovered from Anorexia Nervosa ≥ 2 Years (RAN) and Control (C) Women

Resting Energy Expenditure (R^2 = 0.516)

VARIABLE	Coefficient	Standard Error	p-value
Constant	430.426	160.263	0.012
Group*	-31.517	38.752	0.423
Fat Mass (kg)	2.998	3.752	0.431
Lean Body Mass (kg)	13.066	4.010	0.003[1]
Bone Mineral Content (g)	0.120	0.067	0.081[2]

***C = 0; RAN = 1**

[1]Partial Eta^2 = 0.275

[2]Partial Eta^2 = 0.104

8.3.2 Body Satisfaction and Body Fat Mass

Objective: To determine if higher percent fat mass was correlated with lower body satisfaction overall and to determine if there is a relationship between percent fat mass, body satisfaction and recovery from anorexia nervosa.

Body Shape Questionnaire-34 was not normally distributed so it was transformed to its natural log and used in regression equations. Percent body fat did not differ between persons in recovery from anorexia nervosa for 2 or more years (RAN) and control (C) participants (29.8 ± 7.2% and 32.1 ± 6.4%, respectively; $p = 0.330$), but RAN participants had a trend for lower body satisfaction (100 ± 33 v. 81 ± 28, respectively; $p = 0.073$). Using regression analysis we showed a trend for women with higher percent body fat being more dissatisfied with her body **(Table 8-2a)**. Including group in the model improved the model indicating that the relationship between percent body fat and body dissatisfaction was stronger by group **(Table 8-2b)**. Furthermore, when including group in the model, both group and percent fat mass were associated with body satisfaction **(Table 8-2b)**. Looking only at RAN volunteers, length of recovery was not predictive of body satisfaction nor was percent body fat **(Table 8-2c)**.

Table 8-2a Body Satisfaction and Percent Body Fat in Women Aged 18-35

Body Shape Questionnaire-34 ($R^2 = 0.057$)

VARIABLE	Coefficient	Standard Error	p-value
Constant	3.985	0.274	0.000
Percent Fat Mass	0.015	0.009	0.094[1]

[1] Partial $Eta^2 = 0.085$

Table 8-2b Body Satisfaction and Percent Body Fat in Women Recovered from Anorexia Nervosa ≥ 2 Years (RAN) and Control (C) Women

Body Shape Questionnaire-34 ($R^2 = 0.169$)

VARIABLE	Coefficient	Standard Error	p-value
Constant	3.764	0.275	0.000
Group*	0.254	0.110	0.028[1]
Percent Fat Mass	0.018	0.008	0.035[2]

*****C= 0; RAN = 1

[1]Partial $Eta^2 = 0.146$

[2]Partial $Eta^2 = 0.136$

Table 8-2c Body Satisfaction, Percent Body Fat, and Length of Recovery in Women with a History of Anorexia Nervosa

Body Shape Questionnaire-34 ($R^2 = 0.038$)

VARIABLE	Coefficient	Standard Error	p-value
Constant	4.013	0.419	0.000
Percent Fat Mass	0.019	0.012	0.145
Months in Recovery	0.000	0.002	0.889

8.3.3 Cortisol and Substrate Oxidation

Objective: To determine the relationship between highest baseline cortisol level and respiratory quotient (RQ) in women with a history of anorexia nervosa in recovery for 2 or more years (RAN) compared to control (C) participants.

Using multiple linear regression analyses, RQ was significantly associated with group, but not highest baseline cortisol level when controlling for food quotient (FQ) **(Table 8-3a)**. Further analysis revealed that controlling for body composition only improved the model slightly, but highest baseline cortisol was positively associated with RQ **(Table 8-3b)**. However, RAN was still associated with lower respiratory quotient and the associated between group and RQ ($p = 0.026$) was stronger than that of cortisol and RQ ($p = 0.040$). Fasting cortisol level was not associated with RQ **(Table 8-3c).**

Table 8-3a Respiratory Quotient and Highest Baseline Cortisol Level in Women Recovered from Anorexia Nervosa ≥ 2 Years (RAN) and Control (C) Participants

Respiratory Quotient (R^2 = 0.307)

VARIABLE	Coefficient	Standard Error	p-value
Constant	0.468	0.603	0.446
Group*	-0.050	0.017	0.008[1]
Highest Baseline Cortisol (nM)	0.001	0.000	0.082
Food Quotient	0.476	0.700	0.505

*****C= 0; RAN = 1

[1]Partial Eta^2 = 0.306

Table 8-3b Respiratory Quotient, Highest Baseline Cortisol Level, and Body Composition in Women Recovered from Anorexia Nervosa ≥ 2 Years (RAN) and Control (C) Participants

Respiratory Quotient (R^2 = 0.321)

VARIABLE	Coefficient	Standard Error	p-value
Constant	0.342	0.649	0.605
Group*	-0.042	0.017	0.026[1]
Highest Baseline Cortisol (nM)	0.001	0.000	0.040[2]
Food Quotient	0.524	0.724	0.478
Fat Mass (kg)	0.002	0.002	0.147
Lean Body Mass (kg)	0.001	0.002	0.679

*****C= 0; RAN = 1

[1]Partial Eta^2 = 0.247

[2]Partial Eta^2 = 0.208

Table 8-3c Respiratory Quotient, Fasting Cortisol Level, and Body Composition in Women Recovered from Anorexia Nervosa ≥ 2 Years (RAN) and Control (C) Participants

Respiratory Quotient (R^2 = 0.160)

VARIABLE	Coefficient	Standard Error	p-value
Constant	0.446	0.725	0.547
Group*	-0.045	0.019	0.030[1]
Fasting Cortisol (nM)	0.001	0.001	0.267
Food Quotient	0.448	0.806	0.586
Fat Mass (kg)	0.003	0.002	0.244
Lean Body Mass (kg)	$1.72*10^{-5}$	0.003	0.995

*****C= 0; RAN = 1

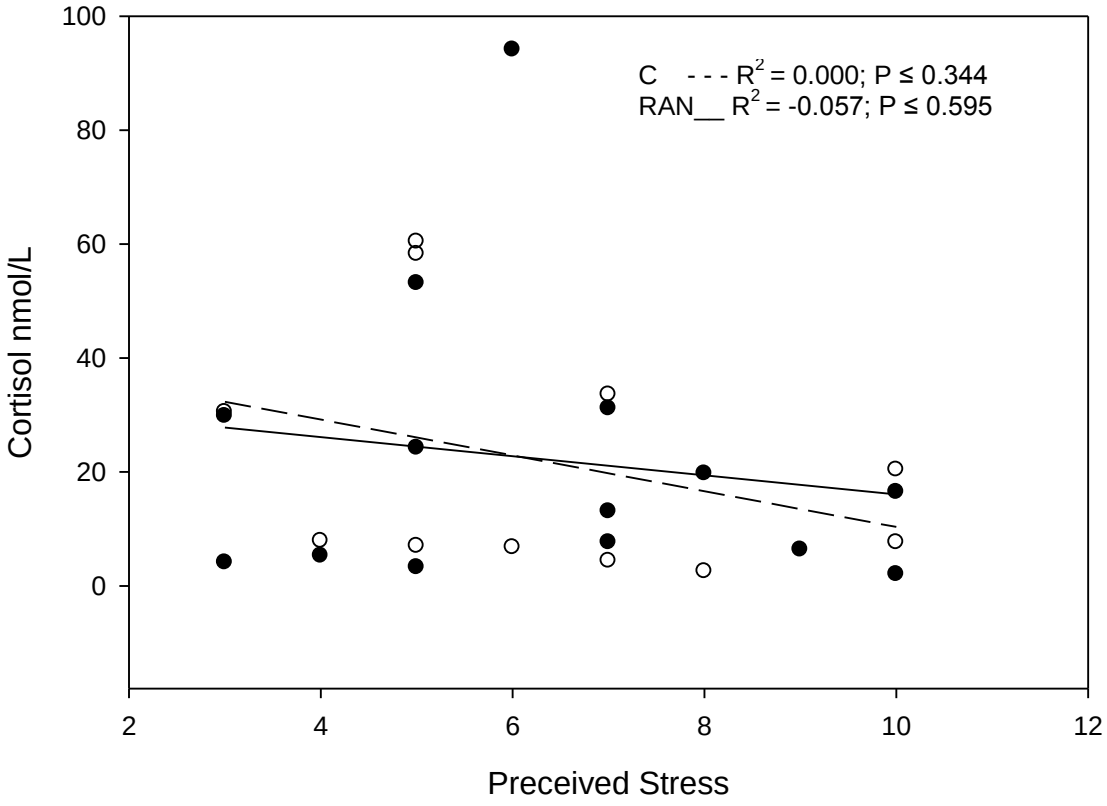
[1]Partial Eta^2 = 0.247

8.3.4 Perceived Stress and Cortisol Levels

Objective: To determine the relationship between perceived stress during the Trier Social Stress Test (TSST) and cortisol levels in women with a history of anorexia nervosa in recovery for 2 or more years (RAN) compared to control (C) participants.

All women were asked on a scale of 1 to 10 with 1 being no stress at all and 10 being the higher stress imaginable, how stressed she was feeling. Subjective stress rating immediately following the TSST did not differ between RAN and C participants (6.6 ± 2.2 v. 6.4 ± 2.3 ($p = 0.819$), respectively). Highest TSST cortisol levels (ten minutes following the TSST) also did not differ between groups (22.2 nM ± 25.2 v. 21.8 nM ± 21.3 ($p = 0.966$), respectively). Cortisol was not normally distributed so its natural log was used for regression analyses. **Figure 8-1** shows the relationship between perceived stress and corresponding cortisol level. There was no interaction between perceived stress level and group ($p = 0.789$) and the relationship did not differ by group.

Figure 8-1 Relationship between Perceived Stress during the TSST and Corresponding Cortisol Levels in Women Recovered from Anorexia Nervosa ≥ 2 Years (RAN●) and Control (C○) Participants



8.3.5 Perfectionism and Cortisol Response to Stress

Objective: Perfectionism has been shown to be related to cortisol response to stress in men using the TSST as the source of stress (222). Here we sought to determine the relationship between perfectionism and three components of perfectionism (perfectionstic self-promotion, nondisplay of imperfection, and nondisclosure of imperfection) all measured by the Perfectionistic Self-Presentation Scale (PSPS) and cortisol response to stress determined by area under the curve (AUC).

AUC was not normally distributed so it was transformed into its natural log and used in multiple linear regression models. We found that AUC was not associated with overall perfectionism ($p = 0.876$). **Figure 8-2** illustrates the relationship between AUC and perfectionism. While group improved the model slightly, the relationship did not differ significantly by group ($p = 0.898$). Perfectionstic self-promotion, nondisplay of imperfection, and nondisclosure of imperfection scales were not associated with AUC **(Table 8-4a-c).**

Figure 8-2 Relationship between Perfectionism and Cortisol Stress Response in Women Recovered from Anorexia Nervosa ≥ 2 Years (RAN●) and Control (C○) Participants

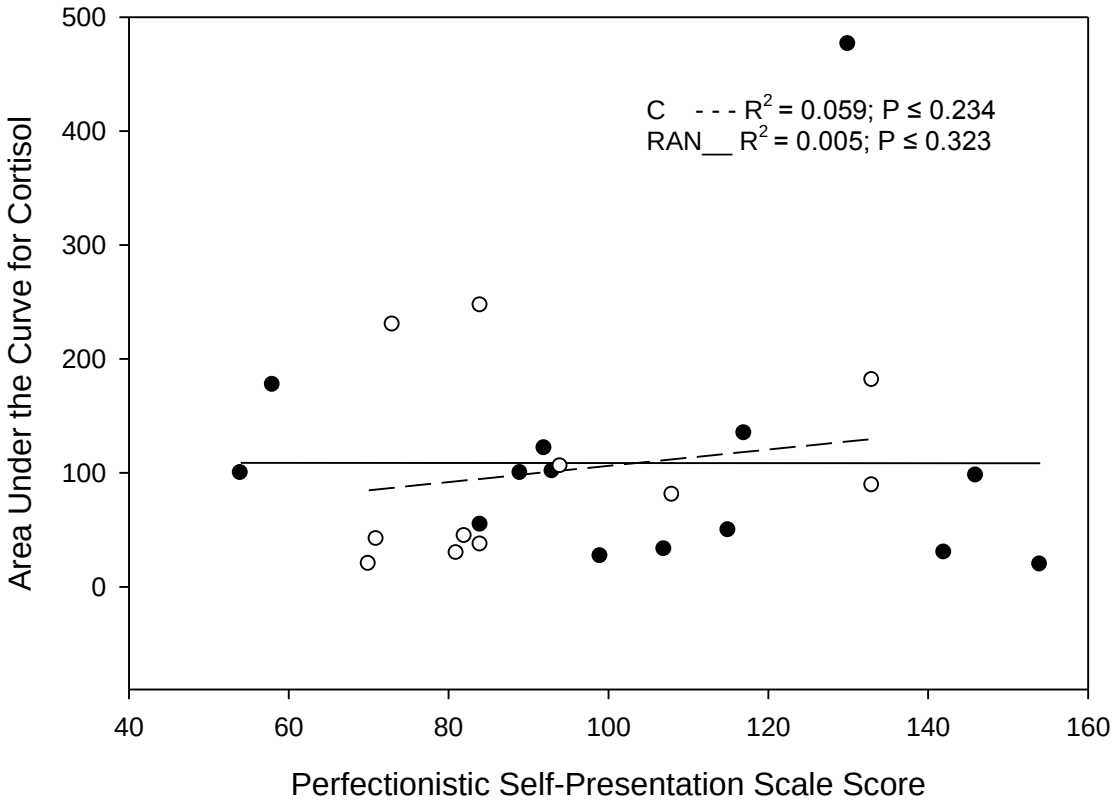


Table 8-4a Cortisol Stress Response and Perfectionstic Self-Promotion in Women Recovered from Anorexia Nervosa ≥ 2 Years (RAN) and Control (C) Participants

Area Under the Curve for Cortisol (R^2 = -0.088)

VARIABLE	Coefficient	Standard Error	p-value
Constant	4.169	0.614	0.000
Group*	0.013	0.364	0.972
Perfectionstic Self-Promotion	0.003	0.015	0.828

***C= 0; RAN = 1

Table 8-4b Cortisol Stress Response and Nondisplay of Imperfection in Women Recovered from Anorexia Nervosa ≥ 2 Years (RAN) and Control (C) Participants

Area Under the Curve for Cortisol (R^2 = -0.082)

VARIABLE	Coefficient	Standard Error	p-value
Constant	4.508	0.591	0.000
Group*	0.048	0.356	0.894
Nondisplay of Imperfection	-0.006	0.014	0.684

***C= 0; RAN = 1

Table 8-4c Cortisol Stress Response and Nondisclosure of Imperfection in Women Recovered from Anorexia Nervosa ≥ 2 Years (RAN) and Control (C) Participants

Area Under the Curve for Cortisol (R^2 = -0.087)

VARIABLE	Coefficient	Standard Error	p-value
Constant	4.430	0.562	0.000
Group*	0.071	0.381	0.855
Nondisclosure of Imperfection	-0.007	0.026	0.781

***C = 0; RAN = 1

8.3.6 Substrate Oxidation and Recovery from Anorexia Nervosa

Objective: To determine if magnitude of undernutrition, indicated by lowest adult Body Mass Index (BMI), is related to substrate oxidation. Further, we explored the relationship between length of recovery and substrate oxidation in women recovering from Anorexia Nervosa for 2 or more years (RAN).

Lowest adult BMI was not associated with RQ value when controlling for food quotient (FQ) in RAN women **(Table 8-5a)**. Further, length of time in recovery from AN did not have a significant impact on RQ when controlling for FQ **(Table 8-5b)**.

Table 8-5a Respiratory Quotient and Lowest Adult Body Mass Index (BMI) in Women with a History of Anorexia Nervosa

Respiratory Quotient (R^2 = -0.129)

VARIABLE	Coefficient	Standard Error	p-value
Constant	0.531	0.830	0.533
Lowest Adult BMI	0.001	0.005	0.833
Food Quotient	0.314	1.008	0.740

Table 8-5b Respiratory Quotient, Lowest Adult Body Mass Index (BMI) and Length of Recovery in Women with a History of Anorexia Nervosa

Respiratory Quotient (R^2 = -0.223)

VARIABLE	Coefficient	Standard Error	p-value
Constant	0.530	0.865	0.551
Lowest Adult BMI	0.001	0.006	0.839
Food Quotient	0.314	1.049	0.751
Months in Recovery	$5.47*10^{-6}$	0.000	0.983

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10 CURRICULUM VITAE

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