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Association of plasma hormones, nutritional status, and stressful life events in anorexia nervosa patients

Związek między osoczowymi stężeniami hormonów, stanem odżywienia i wydarzeniami stresogennymi u pacjentek z jadłowstrętem psychicznym

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Summary

Objective: The aim of the current study was to analyze the relationships between plasma hormones, body weight parameters and stressful life events in anorexia nervosa (AN).

Material and Methods: 72 females in the active phase of AN were evaluated. 52 healthy women constituted the control group. RIA kits were used to measure plasma hormone levels.

Results: The concentrations of leptin, insulin, IGF-1, triiodothyronine, LH, FSH, estradiol, and testosterone were significantly lower and those of cortisol and growth hormone significantly higher in the AN than the control group. No hormonal differences between restrictive and binge-purging AN subtypes were found. Leptin, IGF-1, gonadotropins, and sex steroids correlated significantly negatively and growth hormone positively with total reduction of body weight or the degree of undernutrition. Associations were also found between lower insulin concentration and family violence, lower cortisol and psychiatric diseases in the family, higher testosterone and patient's alcohol or drug abuse.

Discussion: The changed activity of the somatotropin-somatomedin, gonadal, and corticotrophin axes corresponds to the clinical stage of AN. Plasma IGF-1 seems to be the most sensitive and useful independent hormonal marker of cachexia.

Key words: anorexia • hormones • IGF-1 • life circumstances • nutritional status

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Abbreviations: **AN** – anorexia nervosa, **AN-B** – bulimic type of anorexia, **AN-R** – restrictive type of anorexia, **BMI** – body mass index, **B.w.** – body weight, **C** – cortisol, **E_2** – estradiol, **FSH** – follicle-stimulating hormone, **fT_3** – triiodothyronine, **fT_4** – thyroxine, **GH** – growth hormone, **IGF-1** – insulin-like growth factor 1, **IRI** – insulin, **LEP** – leptin, **LH** – luteotropic hormone, **SD** – standard deviation, **T** – testosterone, **TRBW** – total reduction of body weight, **TSH** – thyroid-stimulating hormone, **TTI** – total time of illness, **%IBW** – percentage of ideal body weight.

Introduction

It is still uncertain whether the psychiatric and/or endocrinal disturbances in anorexia nervosa (AN) are the results of a primary hypothalamic disorder or are indicative of longstanding malnutrition [5,51]. Body weight appears to be a crucial determinant of such dysregulation [5,11]. Puberty may itself be a risk factor of AN onset, which is why this disease most commonly develops during adolescence or young adulthood [28]. The European Symptom Checklist For Mental Disorders (ICD–10) describes disturbances of multiple hormonal axes in anorexia [24]. Amenorrhea is one of the main AN diagnostic criteria, which also include disturbed body image, fear of obesity, and aiming at minimal weight even when significantly underweight [1,24]. The American Psychiatric Association (DSM-IV) distinguishes the restricting subtype where low weight is maintained largely by caloric restriction and the bingeing/purging subtype with both binge eating and purging by vomiting, use of laxatives, diuretics, enemas and exercise [1]. Hypoleptinemia is believed to be the major signal underlying both somatic and behavioral adaptation to starvation [37]. Leptin, an adipose tissue-derived adipocytokine, contributes to mechanisms responsible for body weight and energy balance regulation [19,54]. Receptors for leptin have been found in the brain and peripheral glands and it acts as a negative-feedback signal [15]. A certain threshold of leptin is necessary to activate the hypothalamic-pituitary-gonadal and thyroid axes, whereas the hypothalamic-pituitary-adrenal and growth hormone (GH)-insulin-like growth factor 1 (IGF-1) axes may be largely independent of circulating leptin levels in humans [6,35,38]. On the other hand, hypoleptinemia may be subsequent to the decreased nutritional state and predicts secretion of estradiol, thyroid hormones, cortisol, and GH [35]. The role of leptin in abnormal eating behavior is unclear [38].

The recent literature focuses on the possible role of the hormones, severe life events and chronic stress as factors capable of influencing the onset and the course of eating disorders [30]. These psychoendocrine associations might be important for multidisciplinary and complementary AN treatment. The aim of the current study was to explore the relationships between selected clinical features, environmental (familiar) stressors and plasma concentrations of a broad spectrum of hormones involved in psychoendocrine reaction to prolonged starvation in patients with an active stage of anorexia nervosa.

Materials and Methods

Participants

The study group consisted of 72 females aged 20.7 ± 3.5 years, admitted to the inpatient and outpatient services of the Psychiatry and Endocrinology Departments of Pomeranian Medical University in Szczecin from 1997 to 2007. All the patients met the criteria for anorexia nervosa of the Diagnostic and Statistical Manual for Mental Disorders, 4th ed., rev. (DSM-IV) [1], and the International Classification of Mental Disorders and Behavioral Disturbances, 10th ed., rev. (ICD-10) [24]. Forty-nine patients had the restrictive type of anorexia (AN-R) and 23 were bulimic (AN-B). Before blood sampling, all the participants were evaluated by a semi-structured clinical interview (SCID) for current or prior psychiatric illnesses suffered either by them or their first-degree relatives and their environmental situation (negative life events in patient's life up to the moment of gathering data), alcohol or other substance abuse, and self-destructive behavior. The control group was composed of 52 healthy women who were mostly a representative group of medical students with an average age of 27.1 ± 6.3 years, with no history of an eating disorder or any substance abuse. None of the participants had received medication or hormonal therapy for a period of at least four weeks before the experiment. They were free of any somatic diseases such as primary endocrine, autoimmune, and gastrological disorders. Neurological processes that might cause central nervous system deficits were also excluded as well as pregnancy. All the participants received verbal and written information from the investigators about the purpose and design of the study. Informed consent to participate in this study was obtained from each participant and from the parents of the minors taking part in it. The experimental protocols were reviewed and approved by the Ethics Committee of the Pomeranian Medical University of Szczecin.

Hormone assays

Blood samples were collected in the early morning after an overnight fast and rest. In menstruating women a sample for hormonal measurements was collected in the follicular phase (6-9th day of the cycle). Plasma was stored at -80ºC until assayed. Specific radioimmunoassay (RIA) kits were used to measure plasma levels of leptin (LEP) (Linco Research, Inc., St. Louis, MO, USA), cortisol (C)

Table 1. Clinical parameters of the anorexia (AN) group with division into restrictive (AN-R) and bulimic (AN-B) subtypes.

Group	AN-R (N=49)	AN-B (N=23)	p
Parameter	Mean ± SD or median (IQR)		
Age [years]	20 (3)	20 (6)	0.93
TTI [months]	24 (36)	36 (60)	0.44
B.w. [kg]	40.3 ± 6.3	42.6 ± 5.2	0.12
TRBW [kg]	18 (9)	17.8 (9)	0.76
BMI [kg/m2]	14.8 ± 1.7	15.7 ± 1.4	0.037
%IBW [%]	65.0 (9.2)	67.0 (9.8)	0.19

Mean ± SD and p-value of Student t test are shown for normally distributed variables while median (interquartile range – IQR) and p-value of Mann-Whitney test are presented for variables with distributions different from normal; TTI – total time of illness, B.w. – body weight, TRBW – total reduction of body weight, BMI – body mass index, %IBW – percentage of ideal body weight

(Orion Diagnostica, Espoo, Finland), insulin-like growth factor 1 (IGF-1) (BioSource Europe S.A., Belgium), triiodothyronine and thyroxine (fT_3, fT_4) (ZenTech, ANGLEUR Belgium), estradiol (E_2) (BioSource Europe S.A., Nivelles, Belgium), and testosterone (T) (Orion Diagnostica, Espoo, Finland). Plasma insulin (IRI), thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH), and luteotropic hormone (LH) were measured by immunoradiometric assay (IRMA) kits (BioSource Europe S.A., Nivelles, Belgium) and human growth hormone (GH) by immunoradiometric assay (GH-IRMA) kits (BioSource Europe S.A., Nivelles, Belgium). The sensitivities (the lower limits of detection) and intra- and interassay coefficients of variation were: 0.5 ng/mL, 3.4-8.3% and 3.0-6.2% for LEP; 5 nmol/L, 2.6-5.4% and 6.5-7.3% for C; 0.4 µg/L, 3.9-9.5% and 5-8% for IGF-1; 1.7 pmol/L, 3.6-4.2% and 3.4-4.8% for fT_4; 17.6 pmol/L, 5.3-5.8% and 6.8-9.5% for E_2; 0.1 nmol/L, 3.8-7.5% and 4.8-7.0% for T; 1 mIU/L, 1.6-2.2% and 6.1-6.5% for IRI; 0.025 mIU/L, 0.6-6.0% and 2.1-4.1% for TSH; 0.1 IU/L, 1.5-2.7% and 2.0-5.3% for FSH; 0.07 IU/L, 1.0-5.0% and 3.3-5.7% for LH; 0.026 mIU/L for GH, respectively.

Nutritional status

Both the total time of illness (TTI, months) and total reduction of body weight (TRBW, kg) were determined. Some indices of body weight were also calculated, i.e. the body mass index (BMI) as the ratio of body weight [kg] to [height]^2 and %IBW (percentage of ideal body weight) as the ratio of actual to ideal body weight (IBW) x 100%, where IBW (kg) = height (cm) - 100 - {[(height (cm) – 150)]/2} according to Lorentz's formula [31].

Statistical analysis

The normality of distribution of quantitative variables was checked with the Shapiro-Wilk test. The statistical evaluation of differences between the groups was performed by means of Student t test for normally distributed variables or nonparametric Mann-Whitney test when the distributions were significantly different from normal. Associations between quantitative variables were characterized by Spearman's rank correlation coefficient. The general linear model (GLM) was used for multivariate analysis. Statistica 7.1 software was used for statistical calculations. The results are presented as the mean ± SD (standard deviation) for normally distributed variables and as median (interquartile range) for non-normally distributed ones. The level of statistical significance was set at $p<0.05$ and it was not corrected for multiple testing.

Results

There was no significant association between the degree of undernutrition (TRBW, %IBW) and the type of anorexia, but BMI was lower in the restrictive type (Table 1).

The hormone concentrations in the AN-R and AN-B subgroups showed no significant differences (Table 2). Therefore we combined these subgroups and further analyses refer to the whole anorectic group. The anorectic and control groups were significantly different with regard to age, body weight (b.w.), and BMI (Table 3). The concentrations of LEP, IRI, IGF-1, fT_3, LH, FSH, E_2, and T were significantly lower and those of C and GH significantly higher in the AN group than in the controls (Table 3).

The associations of social factors and stressful life circumstances with clinical features and hormone concentrations in the patients with anorexia were explored and those with statistical significance are presented in Table 4. The patients with parental alcoholism were significantly older and had higher E_2 and lower C values, while those who abused alcohol themselves had higher T concentrations. Drug abuse was associated with younger age and higher values of body weight, BMI, and T concentration. Patients with a history of parental separation or divorce had higher BMI. Lower concentrations of IRI were observed in patients with domestic violence, while the subgroup with psychological abuse had lower FSH. Patient's competition with the mother was associated with a longer time of illness, while such behavior towards sib-

Table 2. Hormonal characteristics of the anorexia nervosa patients stratified into restrictive (AN-R) and bulimic (AN-B) subtypes

Hormone in plasma	AN-R		AN-B		p
	N	Mean ± SD or median (IQR)	N	Mean ± SD or median (IQR)	
LEP [ng/mL]	44	1.81 (1.90)	19	1.90 (1.56)	0.38
C [nmol/L]	43	623.0 ± 164.6	19	636.5 ± 124.0	0.75
IRI [pmol/L]	42	28.34 (15.79)	19	31.57 (25.12)	0.35
IGF-1 [µg/L]	48	203.7± 76.2	23	212.0 ± 99.8	0.70
GH [pmol/L]	47	62.17 (130.44)	23	30.23 (79.19)	0.22
TSH [mIU/L]	42	1.49 (1.33)	19	1.12 (1.16)	0.24
fT_3 [pmol/L]	42	4.34 (1.25)	19	4.45 (1.70)	0.53
fT_4 [pmol/L]	42	15.36 (8.88)	19	13.0 (6.57)	0.06
LH [IU/L]	47	1.30 (2.72)	23	1.53 (4.40)	0.33
FSH [IU/L]	47	3.74 (4.60)	23	3.50 (6.09)	0.97
E_2 [pmol/L]	47	68.4 (78.69)	23	67.70 (112.30)	0.71
T [nmol/L]	39	1.59 ± 0.72	18	1.32 ± 0.76	0.20

Mean ± SD and p-value of Student t test are shown for normally distributed variables while median (interquartile range – IQR) and p-value of Mann-Whitney test are presented for variables with distributions different from normal; LEP – leptin, C – cortisol, IRI – insulin, IGF-1 – insulin-like growth factor 1, GH – growth hormone, TSH – thyroid-stimulating hormone, fT3 – triiodothyronine, fT4 – thyroxine, LH – luteotropic hormone, FSH – follicle-stimulating hormone, E2 – estradiol, T – testosterone

Table 3. Characteristics of the anorexia nervosa (AN) and control (C) groups

Parameter	AN		C		p
	N	Mean ± SD or median (IQR)	N	Mean ± SD or median (IQR)	
Age [years]	72	20 (5)	52	25 (2)	<0.00001
B.w. [kg]	72	41.0 ± 6.0	52	57.5 ± 6.4	<0.00001
BMI [kg/m^2]	72	15.1 ± 1.7	52	20.7 ± 1.8	<0.00001
LEP [ng/mL]	63	1.90 (1.80)	52	6.31 (4.78)	<0.00001
C [nmol/L]	62	627.1 ± 152.4	52	519.4 ± 159.4	0.00037
IRI [pmol/L]	61	29.42 (15.79)	52	41.98 (21.53)	0.00001
IGF-1 [µg/L]	71	206.4 ± 83.9	52	286.8 ± 65.0	<0.00001
GH [pmol/L]	70	55.69 (116.12)	48	7.89 (54.14)	0.00006
TSH [mIU/L]	61	1.28 (1.29)	52	1.73 (1.45)	0.053
fT_3 [pmol/L]	61	4.38 (1.23)	52	5.37 (1.10)	<0.00001
fT_4 [pmol/L]	61	15.06 (8.24)	52	13.71 (4.63)	0.10
LH [IU/L]	70	1.34 (2.88)	49	5.03 (2.54)	<0.00001
FSH [IU/L]	70	3.60 (5.03)	49	5.96 (2.11)	<0.00001
E_2 [pmol/L]	70	68.05 (81.63)	50	177.45 (131.30)	<0.00001
T [nmol/L]	57	1.51 ± 0.74	50	1.86 ± 0.82	0.020

Mean ± SD and p-value of Student t test are shown for normally distributed variables while median (interquartile range – IQR) and p-value of Mann-Whitney test are presented for variables with distributions different from normal; B.w. – body weight, BMI – body mass index, LEP – leptin, C – cortisol, IRI – insulin, IGF-1 – insulin-like growth factor 1, GH – growth hormone, TSH – thyroid-stimulating hormone, fT3 – triiodothyronine, fT4 – thyroxine, LH – luteotropic hormone, FSH – follicle-stimulating hormone, E2 – estradiol, T – testosterone

Table 4. Clinical features and plasma hormone concentrations according to environmental stress events in AN patients

Stress event	Analyzed parameters	Patients without stress event		Patients with stress event		p
		N	Mean ± SD or median (IQR)	N	Mean ± SD or median (IQR)	
Parental alcoholism	Age [years]	35	19 (3)	37	21 (5)	0.046
	C [nmol/L]	32	668 ± 132	30	583 ± 163	0.027
	E_2 [pmol/L]	34	50.7 (50.3)	36	93.4 (73.3)	0.011
Alcohol abuse by patient	T [nmol/L]	51	1.44 ± 0.74	6	2.08 ± 0.41	0.045
Drug abuse by patient	Age [years]	66	21 (4)	6	18 (2)	0.017
	B.w. [kg]	66	40.5 ± 6.0	6	46.5 ± 2.3	0.00028
	BMI [kg/m^2]	66	14.9 ± 1.7	6	16.4 ± 0.6	0.00019
	T [nmol/L]	52	1.43 ± 0.73	5	2.33 ± 0.15	<0.00001
Domestic violence	IRI [pmol/L]	31	32 (29)	30	28 (22)	0.004
Psychological abuse	FSH [IU/L]	37	3.9 (3.9)	33	2.8 (4.3)	0.045
Parental separation/divorce	BMI [kg/m^2]	44	14.7±1.8	27	15.6±1.4	0.026
Competition with mother	TTI [months]	57	24 (36)	15	48 (84)	0.038
Competition with siblings	LH [IU/L]	39	2.4 (3.7)	31	1.0 (1.1)	0.013
	FSH [IU/L]	39	4.8 (5.2)	31	3.0 (3.6)	0.017
	E_2 [pmol/L]	39	79.5 (9.1)	31	48.9 (73.4)	0.041
Psychiatric diseases in the family	C [nmol/L]	46	661±130	16	530±175	0.0024

p – statistical significance of difference between patients with and without indicated environmental stress event. Only associations with $p<0.05$ are shown. Mean ± SD and p-value of Student t test are shown for normally distributed variables while median (interquartile range – IQR) and p-value of Mann-Whitney test are presented for variables with distributions different from normal; C – cortisol, E_2 – estradiol, T – testosterone, B.w. – body weight, BMI – body mass index, IRI – insulin, FSH – follicle-stimulating hormone, TTI – total time of illness, LH – luteotropic hormone

lings correlated with significantly lower LH, FSH, and E_2 concentrations. In patients with a history of psychiatric disease in the family, the concentration of cortisol was lower than in those free of this stressor.

The correlations between clinical features and nutritional status parameters on the one hand and hormone concentrations on the other in the patients and controls are presented in Table 5. In the AN group the strongest associations concerned IGF-1, which correlated positively with body weight, %IBW, and BMI (Figure 1) and negatively with TRBW. Similar but much weaker associations were observed for leptin, LH, FSH, E_2, and T. fT_4 correlated positively only with TTI. In contrast to the other hormones, GH had a negative correlation with %IBW and positive with TRBW. The correlations of IRI and TSH and fT_3 with body weight parameters were not statistically significant.

In the controls, negative correlations between fT_3 and body weight and BMI were found, while age correlated positively with E_2 and negatively with IGF-1 and fT_3 (Table 5). The other hormones did not significantly correlate with body weight parameters in this group.

Correlation between plasma hormone concentrations in the AN patients are presented in Table 6.

Multivariate analysis adjusted for patient age, total time of illness, and type of anorexia (restrictive or binge-purging) revealed that high IGF-1 concentration was a strong independent predictor of higher BMI, %IBW, and lower TRBW (Table 7).

Discussion

Multiple endocrine and metabolic changes are involved in the adaptation to survive prolonged starvation in the course of anorexia nervosa. To define these mechanisms it is necessary to sum the numerous pathways, as even minor weight changes in AN are associated with significant responses in basal and dynamic hormonal secretion [11,51]. As regards age and duration of illness in anorectic subgroups, no statistically significant differences were confirmed in our data, similarly to other authors [12], although the patients with bulimia had a slightly higher (by 0.9 kg/m^2) BMI value. This difference could be explained by different principles and rituals pattern in AN-R and AN-B behavior according to anorexia diagnostic criteria. Patients with AN-B who have sig-

Table 5. Spearman rank correlation coefficients for clinical features and plasma hormone concentrations in the AN patients and the control group

Group	AN						Control		
	Age	TTI	B.w.	TRBW	%IBW	BMI	Age	B.w.	BMI
LEP	-0.08	-0.18	+0.18	-0.27*	+0.12	+0.30*	+0.17	+0.01	+0.17
C	+0.12	+0.10	+0.08	-0.13	+0.24	+0.16	-0.04	+0.04	-0.02
IRI	0.00	+0.08	-0.01	-0.13	+0.01	-0.02	+0.05	0.00	+0.09
IGF-1	-0.20	-0.13	+0.47***	-0.42***	+0.48***	+0.57***	-0.37**	-0.26	-0.26
GH	-0.03	-0.11	-0.06	+0.26*	-0.29*	-0.10	-0.02	-0.01	+0.07
TSH	+0.14	+0.05	+0.15	-0.05	+0.04	+0.16	-0.01	-0.21	-0.14
fT_3	+0.2	+0.12	+0.16	-0.06	+0.25	+0.10	-0.31*	-0.32*	-0.29*
fT_4	+0.11	+0.27*	+0.17	0.00	+0.15	-0.05	+0.14	+0.07	-0.05
LH	+0.17	+0.18	+0.21	-0.20	+0.30*	+0.27*	+0.09	-0.09	-0.04
FSH	+0.06	+0.29*	+0.05	-0.26*	+0.24*	+0.23	-0.07	-0.20	-0.27
E_2	+0.15	+0.33**	+0.22	-0.12	+0.35**	+0.21	+0.31*	+0.19	+0.14
T	-0.01	-0.05	+0.40**	-0.20	+0.22	+0.33*	+0.17	+0.10	+0.13

*p<0.05, **p<0.01, ***p<0.001

TTI – total time of illness, B.w. – body weight, TRBW – total reduction of body weight, %IBW – percentage of ideal body weight, BMI – body mass index, LEP – leptin, C – cortisol, IRI – insulin, IGF-1 – insulin-like growth factor 1, GH – growth hormone, TSH – thyroid-stimulating hormone, fT_3 – triiodothyronine, fT_4 – thyroxine, LH – luteotropic hormone, FSH – follicle-stimulating hormone, E_2 – estradiol, T – testosterone

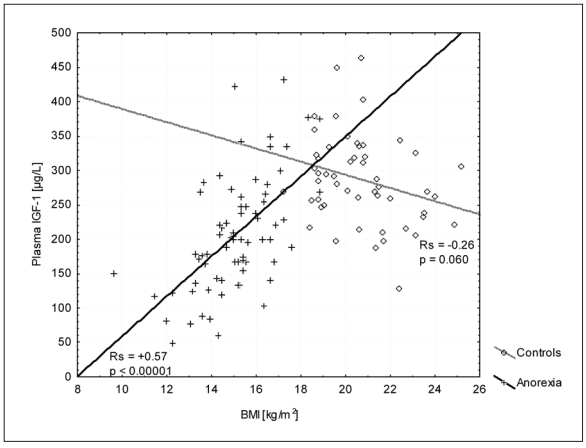

Fig. 1. Correlations of IGF-1 plasma concentration and BMI in the AN patients and controls. Linear regression lines and Spearman's rank correlation coefficients (Rs) are presented for each group

nificantly higher BMI in comparison to AN-R are more prone to develop bulimia nervosa later [40].

Our observations show that AN-R and AN-B patients have similar hormonal profiles and these data may suggest that from the metabolic point of view they suffer from the same disease. We confirmed total leptin secretion being significantly lower, both in the restrictive and binge eating/purging AN subtypes, than in the normal-weight women [8,36,38]. A negative correlation between leptin and TRBW and a positive correlation between leptin and BMI were found in the AN patients in our study. Some authors observed no significant correlations between leptin, BMI, and body weight values in AN [42], while others assumed that the correlation between leptin and BMI was preserved independently of any specific pathology [13]. Serum leptin concentration corresponds to the true status of adipose stores and is supposed to be responsible for changes in the neuroendocrine system [8,39]. Leptin seems to be a link between the central neuropeptides and the hypothalamus–pituitary–peripheral hormones [48]. The central anorectic role of leptin is believed to inhibit the anabolic (orexigenic) pathway connected with such agents as AgRP (Agouti-related protein), NPY (neuropeptide Y), β-EP (β-endorphin), MCH (melanin-concentrating hormone), galanin, hypocretins (OXA, OXB), glutamate and NMDA [48]. On the other hand, leptin seems to stimulate a catabolic (anorexigenic) pathway of the central nervous system connected with CRH (corticotrophin-releasing hormone), TRH (thyrotropin-releasing hormone), POMC (proopiomelanocortin), α-MSH (α-melanocortin), CART (cocaine- and amphetamine-regulated transcript), and BDNF (brain-derived neurotrophic factor) [21,48,49]. TNFα could also contribute to the hormonal changes and to the loss of appetite due to interaction with leptin and NPY signaling [48]. Leptin with its receptor (LepRb) and

Table 6. Spearman rank correlation coefficients for plasma hormone concentrations in the AN patients

	LEP	C	IRI	IGF-1	GH	TSH	fT_3	fT_4	LH	FSH	E_2
C	-0.09										
IRI	+0.27*	-0.03									
IGF-1	+0.43***	+0.25	+0.18								
GH	-0.11	-0.14	-0.42***	-0.11							
TSH	-0.06	+0.15	-0.06	+0.17	-0.07						
fT_3	-0.06	+0.14	+0.07	+0.17	-0.31*	+0.18					
fT_4	-0.25*	+0.06	+0.17	+0.08	-0.27*	-0.02	+0.33**				
LH	+0.32*	+0.07	+0.16	+0.35**	-0.12	+0.18	+0.27*	+0.13			
FSH	+0.28*	-0.13	-0.07	+0.26*	+0.02	+0.03	+0.08	+0.07	+0.65***		
E_2	+0.36**	-0.11	+0.08	+0.08	-0.10	+0.10	+0.16	+0.16	+0.47***	+0.42***	
T	+0.17	-0.06	+0.11	+0.42**	-0.05	+0.12	+0.13	+0.17	+0.22	+0.16	-0.01

*p<0.05, **p<0.01, ***p<0.001

LEP – leptin, C – cortisol, IRI – insulin, IGF-1 – insulin-like growth factor 1, GH – growth hormone, TSH – thyroid-stimulating hormone, fT_3 – triiodothyronine, fT_4 – thyroxine, LH – luteotropic hormone, FSH – follicle-stimulating hormone, E_2 – estradiol, T – testosterone

Table 7. Multiple linear regression analysis (GLM) of associations between body weight parameters and patients' age, type of anorexia (restrictive or binge-purging), total time of illness, and IGF-1 plasma concentration

Dependent variable	Independent variables	Partial correlation coefficient	p
BMI	Age	+0.045	0.72
	Bulimic AN type	+0.254	0.037
	TTI	+0.018	0.88
	IGF-1	+0.588	<0.00001
%IBW	Age	+0.038	0.76
	Bulimic AN type	+0.143	0.24
	TTI	+0.173	0.16
	IGF-1	+0.516	<0.00001
TRBW	Age	+0.114	0.35
	Bulimic AN type	+0.074	0.55
	TTI	-0.110	0.37
	IGF-1	-0.360	0.0025

p – statistical significance of association with the dependent variable for each independent variable in the multivariate model; BMI – body mass index, %IBW – percentage of ideal body weight, TRBW – total reduction of body weight, TTI – total time of illness, IGF-1 – insulin-like growth factor 1

the melanocortin system with MC4R signaling in neural and physiological pathways may be crucial for processes of nutritional regulation [45].

The cortisol level in the examined anorectic women were higher than in the controls. Our study confirmed the opinion that cortisol is usually elevated in anorexia [20,23,26,32,34,41]. According to some authors, hypercortisolemia is one of the most characteristic hormonal symptoms in AN [32,41]. Serum cortisol at low body weight is a significant predictor of visceral adipose tissue redistribution after weight gain [32]. On the basis of endocrinological observations, alteration of the CRH -ACTH-cortisol axis in AN is secondary to starvation [51]. Hypersecretion of CRH is a compensatory mechanism counteracting cortisol resistance in feedback regulation [23] which probably is involved in the vicious circle maintaining the emaciated state [51], while a reduced rate of

cortisol breakdown might be responsible for energy conservation [10]. We did not confirm a previously reported inverse correlation between cortisol and BMI in AN patients [34,41]. We observed lower cortisol secretion in anorectic patients with psychiatric disease in the family. According to a pathophysiological model, severe stressors or increased plasma cortisol might trigger a host of harmful (brain or peripheral tissue) derangements [22]. This especially concerns individuals carrying a genetic risk for psychiatric disorders such as anorexia nervosa, alcoholism, and affective disorders [22,25]. In anorexia nervosa, neuropsychological deficits are usually linked to elevations in cortisol level, while the structural abnormalities in the brain are similar to those seen in schizophrenic patients [28] who are impaired in their biological response to stress by showing a blunted cortisol secretion to psychosocial stress [17]. Up to 90% of AN patients demonstrate depressive symptoms during the acute phase of illness [28].

The level of insulin was lower in the AN than in the control women. Such results are typical of chronic starvation [2]. No correlations between IRI and the clinical parameters were found except for the association of IRI and domestic violence. Some authors assume that women who have experienced parental physical and psychological abuse demonstrate more anorexic behavior [33,43]. However, complete suppression of appetite is doubtful because of the underlying volitional and, usually, egosyntonic resistance to eating drives [25]. On the other hand, proper parenthood with encouragement of child autonomy is associated with less dieting behavior, possibly serving a protective function against eating disorders [43]. Family violence is an extremely powerful trigger which may lead to activation of the glucose-insulin-cortisol-related pathways in autonomic response to psychological distress and requires further exploration.

IGF-1 was significantly lower and GH significantly higher in the AN group than in the controls and comparable in the AN-R and AN-B subgroups [11,50]. Adaptation to chronic starvation induces exaggerated GH and decreased IGF-1 secretion. Such changes are explained by disturbed control of hypothalamic GH release [46,47,50,51], peripheral GH resistance [47,51], and decreased levels of growth hormone-binding protein (GHBP) [30], leading to reduced IGF-1 synthesis [16] and impairment of the negative IGF-1 feedback action on GH release [16,52]. However, the peripheral IGF-1 system may be independent of any modifications in GH concentration [3], so that some anorectic patients may have diminished GH despite decreased IGF-1 secretion [18]. Our results support the notion that IGF-1 and GH are sensitive to chronic malnutrition [7] as these hormones significantly correlated with TRBW and %IBW. Following Stǿving et al., we demonstrated that IGF-1 plasma level showed the strongest correlations with each of the analyzed body weight parameters irrespective of the clinical subtype of illness [50]. These data are congruent with hypotheses that hormonal changes are secondary to nutritional deprivation [7,18,47] and that nutritional status may be a major determinant in the regulation of the somatotropin-somatomedin axis in humans [2,47]. Bioactivity of IGF-1 may be a suitable marker in emaciated patients with AN [50] as well as in other conditions of malnutrition and malabsorption [16]. On the other hand, these mechanisms connected with increased GH and decreased IGF-1 secretion appear to be protective of the self-destructive stress reaction called self-cannibalism [51], typically observed in long-lasting critical illness [46]. In this context, the positive correlation between IGF-1 and BMI in AN with a tendency to a negative correlation in the control group confirms a close relationship between IGF-1 secretion and nutrition state. In Figure 1 it can be observed that a BMI of 18.5 kg/m^2, which was arbitrarily determined as the boundary for anorexia nervosa diagnosis, is where the IGF-1 levels in the AN and control groups intersect. Thus plasma IGF-1 may be suggested as a sensitive hormonal marker of cachexia. It should be verified whether the level of 300 μg/L IGF-1, which corresponds to a BMI of 18.5 kg/m^2, might be of diagnostic value (Figure 1). Our results may support the hypothesis of Gianotti et al. [16] that the activity of the GH/IGF-1 axis in AN reflects only an impaired nutritional state and cannot be considered a primary hallmark of the disease.

Anorexia nervosa is usually associated with markedly decreased total and free T_3, normal or subnormal total and free T_4, and normal or lower TSH concentrations described as "low T_3 syndrome" or "sick euthyroid syndrome" [27,44]. Our results are in agreement with this profile. The thyroid axis adapts its activity to weight status [44] and leptin is believed to be the link between body weight and TSH [38]. However, no correlation between TSH and body weight parameters was found previously [5] or in our AN patients. Such results may explain the adaptive mechanisms in AN responsible for cell energy expenditure.

Hypogonadotropic hypogonadism, a major diagnostic criterion of AN [41], is a final clinical effect of adaptive responses to pathological dieting and extreme undernutrition [9]. Our results confirm that serum gonadotropins and estradiol in AN (independent of subtype) are significantly decreased compared with the control group [32,50,53]. Our data showing that LH positively correlated with BMI [41,53], and FSH with duration of illness [53], are also in agreement with other reports. These changes are parallel to disturbances in the GH-IGF-1 and corticotrophin axes described above [4,34] and decreased serum levels of leptin, IGF-1, and T_3, which are supposed to be nutritional markers of FHA (functional hypothalamus amenorrhea) [8,39]. Two metabolic determinants are important for primary or secondary amenorrhea. Low fat content in a range of 10-15% body weight is associated with cessation of menstrual cycles [14]. The second clinical determinant is leptin level – its serum concentration of 1.85 ng/mL is required to maintain menstruation [29].

In our study we observed positive associations of testosterone with alcohol and drug abuse in AN patients. Regarding the biological spectrum of testosterone, such observations could explain the proneness to impulsivity in anorexia. As individuals with the binge eating/purging subtype of AN are more often classified as likely to have histories of behavioral dyscontrol, substance abuse, and overt family conflict [25], the similar plasma levels of testosterone in both analyzed subgroups might show the better mechanisms of emotional control in AN-R than in AN-B patients.

A limitation of our study is significantly older age of females in the control group (Table 3). It was connected with ethical constraints in obtaining informed consent for examination of healthy adolescents. However, it is unlikely that the observed differences in hormone concentrations between groups were strongly influenced by an age difference equal to 5 years, and the main aim of our study was to explore the relationships within the anorexia group. Further research is needed to explain the role of many other neurotransmitters and inflammatory agents in AN and their interactions with genetic and environmental factors.

Conclusions

Our study confirmed that there is feedback regulation between nutritional state, leptin, and the concentrations of a broad spectrum of hormones involved in the stress reaction which affect glucose, lipid, and protein metabolism, regulate energy expenditure, and influence reproductive function in women with anorexia nervosa. The somatotropin-somatomedin, gonadal, and corticotrophin axes activity may be helpful in assessing the clinical stage in anorexia nervosa. Plasma IGF-1 seems to be the most sensitive and useful independent hormonal marker of cachexia. The associations of stressful life events, personality predisposition, hormone secretion, eating behavior, and nutritional state are evident and require further exploration to better understand the phenomenon of anorexia nervosa and to help monitor the disease.

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