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Associations between pituitary-thyroid hormones and depressive symptoms in individuals with anorexia nervosa before and after weight-recovery

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ABSTRACT

Background: There is sound evidence that the hypothalamic-pituitary-thyroid axis plays a role in mood regulation. Alterations in this axis, particularly low triiodothyronine syndrome, are a common neuroendocrine adaptation to semi-starvation in patients with anorexia nervosa (AN), who also frequently suffer from co-existing depressive symptoms. We therefore aimed to investigate the associations between pituitary-thyroid function and psychopathology, in particular depressive symptoms, at different stages of AN using a combined cross-sectional and longitudinal study design.

Methods: Pituitary-thyroid status (FT3, free triiodothyronine; FT4, free thyroxine; conversion ratio FT3/FT4; TSH, thyroid-stimulating hormone) was assessed in 77 young acutely underweight females with AN (acAN) and in 55 long-term weight-recovered individuals with former AN (recAN) in a cross-sectional comparison to 122 healthy controls (HC). Further, pituitary-thyroid status of 48 acAN was reassessed after short-term weight-restoration. We performed correlation analyses of pituitary-thyroid parameters with self-reported measures of psychopathology.

Results: AcAN showed significantly lower FT3, FT4, FT3/FT4 ratio, and TSH levels compared to HC. Pituitary-thyroid alterations were partly reversed after short-term weight-restoration. RecAN still had lower FT3 concentrations than HC. Lower FT3 concentrations and FT3/FT4 ratios were associated with more severe depressive symptoms in acAN, occurring prominently in cases of manifest low triiodothyronine syndrome. Longitudinally

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increasing FT3/FT4 ratios (change scores) were inversely correlated with depressive and general psychiatric symptoms after short-term weight-restoration.
Conclusions: Our results suggest a potential modulation of the severity of depressive symptoms by temporarily decreased FT3 concentrations and inhibited thyroid hormone conversion (FT3/FT4 ratios) in acutely underweight AN. Associations between conversion ratios FT3/FT4 and psychopathology seem to persist across short-term weight-restoration. The findings of our study might have relevant clinical implications, ranging from thyroid monitoring to experimental low-dose thyroid hormone supplementation in certain patients with AN showing severe psychiatric impairment and overt thyroid hormone alterations.

1. Introduction

Anorexia nervosa (AN) is a serious eating disorder (ED), typically occurring in adolescent females and characterized by a disturbed body image, an immense fear of weight gain, and, despite extreme emaciation, a perpetual drive for weight loss, mostly by self-starvation (American Psychiatric Association, 2013). The complex neuroendocrine alterations that accompany acute AN have been studied extensively, among them changes in the hypothalamic-pituitary-thyroid (HPT) axis (Lawson and Klibanski, 2008). However, the psychiatric relevance of AN-related HPT axis alterations, such as the frequently observed low triiodothyronine (low-T3) syndrome, is still unclear in the field of AN research.

There is sound evidence for neuropsychiatric, most commonly depressive symptoms to occur with an up to seven-fold increased risk under primary hypothyroidism (Hage and Azar, 2012; Larisch et al., 2004). Illustratively, even in patients with subclinical hypothyroidism, treatment with levothyroxine (LT4) and liothyronine (LT3) preparations may not only normalize thyroid function but also be effective to reduce depressive symptoms (Najafi et al., 2015). Further, major depressive disorder is well-known to occasionally co-occur with secondary thyroid hormone abnormalities like sub-normal triiodothyronine levels (Hage and Azar, 2012). The adjunctive administration of LT3 and supra-physiological doses of LT4 was reported to enhance the efficacy of antidepressant pharmacotherapy (Bauer and Whybrow, 2021). As a physiological rationale behind these observations, the intracerebral availability of HPT axis hormones has been hypothesized to influence the brain's metabolic activity (e.g., in the limbic system), promote serotonergic neurotransmission and affect further neurotransmitter systems like dopamine and norepinephrine with known associations to neuropsychiatric, including emotional conditions (Bauer et al., 2002). However, it remains to be clarified whether and how modulatory impacts of thyroid hormones on mood also play a role in patients with AN. These patients show substantial HPT axis alterations with evident effects on metabolic homeostasis (Estour et al., 2010; Misra et al., 2005; Onur et al., 2005; Reinehr et al., 2008; Swenne et al., 2009) and at the same time severely depressed mood (Zipfel et al., 2015) and imbalances in central serotoninergic and dopaminergic systems (Kaye et al., 2013). Links between these neurobiological and clinical features of AN, possibly comparable to associations between thyroid function and psychopathology in primary hypothyroidism or depression, have rarely been investigated.

Previous studies provided persuasive evidence for significant reductions in total (T3) or free (FT3) triiodothyronine concentrations in patients with AN, referred to as low-T3 syndrome (Table 1(A/B); Andrés-Perpina et al., 2011; Buehren et al., 2011; Laessle et al., 1988; Pahl et al., 1985). Low-T3 syndrome is often combined with borderline-low total (T4) or free (FT4) thyroxine and low-normal thyroid-stimulating hormone (TSH) concentrations in acute AN (Brambilla et al., 2006; Estour et al., 2010; Misra et al., 2005; Onur et al., 2005; Reinehr et al., 2008; ŚmIarowska et al., 2014; Støving et al., 2001; Swenne et al., 2009). Beyond peripheral HPT axis alterations, the lack of overshooting TSH responses in reaction to low FT3 and FT4 concentrations as well as delayed or blunted TSH responses to exogeneous thyrotropin-releasing hormone suggest a central involvement of hypothalamic-pituitary elements of the HPT axis in acute AN, shifting setpoints in the neuroendocrine feedback loop to regulate thyroid function (Leslie et al., 1978; Miyai et al., 1975; Tamai et al., 1986).

Low-T3 syndrome has been stated to evolve mainly from inhibition of the enzymatic deiodination of FT4 into metabolically active FT3, partly caused by elevated cortisol (Lee and Farwell, 2016). Instead, FT4 is increasingly converted into inactive reverse T3 under semi-starvation (Table 1(B); Leslie et al., 1978; Tamai et al., 1986). Assessing the ratio of FT3 to FT4 concentrations measured in peripheral blood samples, i.e., FT3/FT4 ratio, can indirectly address the thyroid hormone conversion deficiency as described above (Yu et al., 2018). FT3/FT4 ratio has been demonstrated to be diminished in patients with AN (Table 1(B); Miyai et al., 1975; Nomura et al., 2017), which resembles euthyroid sick syndrome in critical systemic illness (Lee and Farwell, 2016), where FT3/FT4 ratio serves as an index of clinical prognosis (Pasqualetti et al., 2018; Yu et al., 2018).

HPT axis-related neuroendocrine alterations in AN are commonly characterized as temporary protective adaptations to reduce energy expenditure (Lawson and Klibanski, 2008; Onur et al., 2005), and thus mark the acute state of AN rather than constituting trait markers. In line with that, all aforementioned changes in the HPT axis tend to reverse more or less rapidly during weight-restoration (Table 1(A/B); Buehren et al., 2011; Leslie et al., 1978; Onur et al., 2005; Pahl et al., 1985; Reinehr et al., 2008; Swenne et al., 2009; Tamai et al., 1986).

Previous, predominantly cross-sectional studies on HPT axis hormones and psychopathology in AN have generated inconsistent and often statistically non-significant findings regarding specific HPT axis elements in relation to neuropsychiatric symptom dimensions, most importantly depressive symptoms (Table 1(A); Andrés-Perpina et al., 2011; Brambilla et al., 2006; Laessle et al., 1988; ŚmIarowska et al., 2014). Moreover, the scientific grounds for longitudinal relationships between HPT axis elements and depressive symptoms during weight-restoration of patients with AN are hitherto sparse (Buehren et al., 2011; Pahl et al., 1985). There is a need for more studies on these relationships in larger samples of acute patients with AN and recovered individuals, particularly since illuminating interactions between neurobiological and clinical characteristics of AN is of great scientific interest and might be of future therapeutic importance (Schaumberg et al., 2017).

In the present study, we therefore focused on investigating whether pituitary-thyroid hormone alterations as seen in patients with AN might act as neuroendocrine factors involved in the modulation of co-existing depressive symptoms – not only in acutely underweight AN, but also over the course of short-term weight-restoration and after long-term weight-recovery – in a combined cross-sectional and longitudinal study design. To test our hypothesis that the degree of starvation-induced HPT axis alterations relates to the severity of depressive symptoms in AN, we analyzed associations of FT3, FT4, and TSH concentrations, obtained from fasting plasma samples via a standardized protocol, with a self-reported measure of depressive symptoms (BDI-II; Hautzinger et al., 2009). We also explored associations with AN and general psychiatric symptoms. Conversion ratios of thyroid hormones FT3/FT4 were further examined to achieve a better understanding of the psychiatric relevance of thyroid hormone conversion deficiency in AN. We expected that associations between pituitary-thyroid hormone

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Table 1
Hypothalamic-pituitary-thyroid (HPT) axis research on anorexia nervosa in humans.

(1) Authors, year	(2) Study design	(3) AN group	(4) HPT axis characteristics				(5) Examined relationships (~) involving HPT axis and neuropsychiatric or metabolic characteristics		(6) Blood samples, assays
			(F)T3	(F)T4	TSH	Other	Depressive symptoms	Further characteristics	
(A) Studies on the relationships between HPT axis alterations and neuropsychiatric characteristics in AN									
Andrés-Perpina et al. (2011)	CS	37 (2 M)	$\downarrow^{sn}$ 90%	n/a	n/a	n/a	n/a	$\downarrow$T3 ~ impaired visuospatial performance^{ns}	Samples f, CLIA
Brambilla et al. (2006)	CS	104	$\downarrow$* AN-R/P/BP, EDNOS-AN	$\downarrow$* AN-R/P/BP	$\downarrow$* AN-P, EDNOS-AN	n/a	FT3, FT4, TSH ~ (-)* in EDNOS-AN	FT3, FT4, TSH ~ ED and general psychiatric symptoms$^{(+)\text{ or }(-)*}$	Plasma f, ELISA
Buehren et al. (2011)	CS + LO (4 months)	10	$\downarrow$* at bl, $\uparrow$* Δ, $\downarrow$* at fu	n/a	n/a	n/a	FT3 at bl ~ (-)*	FT3 ~ learning and memory functions^{ns}	Plasma f, ECLIA
Laessle et al. (1988)	CS	14 bl AN; 28 fu AN	$\downarrow^{sn}$ 60% bl AN; $\downarrow^{sn}$ 40% fu AN	n/a	n/a	n/a	ns	n/a	Plasma f, RIA
Pahl et al. (1985)	LO (12 weeks)	22 (1 M)	$\downarrow^{sn}$ 45% at bl, $\rightleftarrows$ 95% at fu	n/a	n/a	n/a	ns	low-T3 at bl ~ $\uparrow$AN symptoms at bl*	Plasma f, RIA
Śmlarowska et al. (2014)	CS	61	$\downarrow$*	$\rightleftarrows$	$\rightleftarrows$	n/a	n/a	FT3, FT4, TSH ~ social burdens or stressful life events (e.g., violence, drug abuse)^{ns}	Plasma f, RIA/IRMA
Støving et al. (2001)	CS	22 (1 M)	$\downarrow$*	$\rightleftarrows$	$\rightleftarrows$	$\downarrow$* thyroid volume (US)	n/a	Thyroid atrophy ~ psychiatric symptoms (only speculative, not tested)	Serum, RIA/DELFIA
Swenne and Rosling (2010)	CS (MDE vs. no MDE)	40 among 239 ED patients	$\rightleftarrows$	$\downarrow$* in MDE ($\downarrow^{sn}$ 1/239)	$\rightleftarrows$	n/a	FT4 ~ (-)*	n/a; note that AN group was not tested individually	Plasma nf, CMIA/CLIA
(B) Studies on HPT axis alterations in AN									
Estour et al. (2010)	CS	210	$\downarrow$*	$\downarrow$*	$\rightleftarrows$	n/a	n/a	FT3, FT4 ~ BMI$^{(+)*}$; low-FT3 ~ $\uparrow$severe medical events*	Plasma f, RIA/CLIA
Leslie et al. (1978)	CS + LO	12	$\downarrow$* at bl, $\uparrow$* Δ	$\downarrow$* at bl, $\rightleftarrows\Delta$	$\rightleftarrows$	$\uparrow$* rT3 at bl	n/a	Delayed/blunted TSH response to TRH at bl	Serum f, RIA
Misra et al. (2005)	CS	23	$\downarrow$*	$\rightleftarrows$	$\rightleftarrows$	n/a	n/a	T3, T4 ~ mean leptin concentration$^{(+)*}$	Serum f, RIA
Miyai et al. (1975)	CS	16	$\downarrow$*	$\downarrow$*	$\rightleftarrows$	$\downarrow$* T3/T4 ratio	n/a	Delayed TSH response to TRH	Serum f, RIA
Nomura et al. (2017)	CS	36 (2 M)	see ratio	see ratio	n/a	$\downarrow$* FT3/FT4 ratio	n/a	FT3/FT4 ratio < 0.24 suggests peripheral low-T3 syndrome (cut-off, FT3 and FT4 in pmol/L)	Serum, CLEIA
Onur et al. (2005)	CS + LO (> 6 weeks)	28 (17 fu)	$\downarrow$* at bl, $\uparrow$* Δ	$\downarrow$* at bl, $\rightleftarrows\Delta$	$\rightleftarrows$	n/a	n/a	FT3 ~ resting energy expenditure$^{(+)*}$	Plasma f, ECLIA
Reinehr et al. (2008)	CS + LO (1 year)	20	$\downarrow$* ($\downarrow^{sn}$ 70%) at bl, $\uparrow$* $\Delta^{\S}$	$\rightleftarrows$	$\downarrow$* at bl, $\uparrow$* $\Delta^{\S}$	n/a	n/a	FT3, TSH at bl ~ BMI at bl$^{(+)*}$; ΔFT3, ΔTSH ~ ΔBMI$^{(+)*}$	Serum f, RIA/CLIA
Swenne et al. (2009)	CS + LO (> 6 weeks)	280 (36 fu)	$\downarrow^{sn}$ 50% at bl, $\uparrow$* Δ	$\downarrow$* or $\rightleftarrows$	$\rightleftarrows$	n/a	n/a	T3, FT4 at bl ~ previous weight loss$^{(-)*}$; T3 ~ BMI-SDS$^{(+)*}$ during treatment	Serum nf, CLIA/DELFIA
Tamai et al. (1986)	CS + LO (several months)	21	$\downarrow$* at bl, $\uparrow$* Δ	$\downarrow$* at bl, $\uparrow\Delta$ or $\downarrow\Delta$	$\downarrow$* at bl, $\uparrow$* Δ	$\uparrow$* rT3 at bl, $\downarrow$* Δ	n/a	Delayed/blunted TSH response to TRH at bl	Serum, RIA

Selected studies on hypothalamic-pituitary-thyroid (HPT) axis alterations (B), and their relationships with neuropsychiatric characteristics (A) in patients with anorexia nervosa (AN) are presented, including: (1) authors and year of publication, (2) study design (CS, cross-sectional; LO, longitudinal; MDE, major depressive episode), (3) characteristics of the AN group with number of included patients (M, male patients (all other patients were female); bl and fu, baseline and follow-up assessments; ED, eating disorder), (4) main results regarding HPT axis characteristics ((F)T3, free or total triiodothyronine; rT3, reverse triiodothyronine; (F)T4, free or total thyroxine; TSH, thyroid-stimulating hormone; arrows indicating HPT axis alterations in AN or group differences: $\downarrow$sn subnormal (i.e., below age-related reference range, percentage or number of patients with AN given), $\downarrow$* significantly lower than healthy control group (or group without MDE (Swenne and Rosling, 2010)), $\rightleftarrows$ unaltered/normal (compared to healthy control group or group without MDE (Swenne and Rosling, 2010)) or no longitudinal change ($\rightleftarrows\Delta$), $\uparrow$* Δ or $\downarrow$* Δ significant longitudinal increase (§ if weight gain > 5%) or decrease; n/a, not assessed; AN subtypes: AN-R, -P, -BP, -restrictive, -purge, -binge/purge; EDNOS, ED not-otherwise-specified (atypical); US, ultrasonography), (5) examined relationships (~) involving HPT axis alterations and depressive symptoms, further neuropsychiatric or metabolic characteristics (n/a, not assessed; ns, non-significant; (+) / (-), positive/negative relationship; *, significant at $p < 0.05$ (exact significance levels are not stated); arrows: $\downarrow$ lower, $\uparrow$ higher; Δ, change score; TRH, thyrotropin-releasing hormone), and (6) types of blood samples and applied hormone assays (f, fasting; nf, non-fasting; (E)CLIA, (electro)chemiluminescence immunoassay; ELISA, enzyme-linked immunosorbent assay; RIA, radioimmunoassay; IRMA, immunoradiometric assay; DELFIA, dissociation-enhanced lanthanide fluorescence immunoassay; CMIA, chemiluminescent microparticle immunoassay; CLEIA, chemiluminescence enzyme immunoassay).

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Table 2
Cross-sectional study sample (acAN-T1, recAN, HC): demographic, clinical, leptin, pituitary-thyroid, and psychiatric symptom characteristics.

	acAN-T1[a] (n 77)	recAN[a] (n 55)	HC[a] (n 122)	ANOVA / ANCOVA[b]	p	Significant group differences (Scheffé post-hoc tests[b])
Demographic and clinical variables						
Age (years)	16.71 (3.61)	22.45 (3.22)	19.09 (4.17)	F(2,251) 36.31 η_p^2 0.22	< .001	acAN < recAN (p < 0.001) acAN < HC (p < 0.001) recAN > HC (p < 0.001)
IQ	112.31 (12.76)	109.29 (8.61)	111.31 (8.94)	F(2,240) 1.40 η_p^2 0.01	0.248	n/a
BMI (kg/m^2)	14.79 (1.38)	20.70 (1.65)	21.15 (2.38)	F(2,251) 267.27 η_p^2 0.68	< .001	acAN < recAN (p < 0.001) acAN < HC (p < 0.001)
BMI-SDS	-3.20 (1.33)	-.54 (0.57)	-.13 (0.67)	F(2,251) 284.74 η_p^2 0.69	< .001	acAN < recAN (p < 0.001) acAN < HC (p < 0.001) recAN < HC (p 0.020)
Minimal lifetime BMI (kg/m^2)	14.34 (1.39)	14.27 (1.54)	19.76 (1.83)	F(2,238) 332.52 η_p^2 0.74	< .001	acAN < HC (p < 0.001) recAN < HC (p < 0.001)
Hormone parameters						
Leptin (µg/L)	1.75 (2.33)	8.85 (5.46)	12.43 (8.35)	F(2,250) 57.27 η_p^2 0.31	< .001	acAN < recAN (p < 0.001) acAN < HC (p < 0.001) recAN < HC (p 0.003)
Pituitary-thyroid characteristics						
FT3 (pmol/L)	3.36 (0.77)	5.07 (0.93)	5.49 (0.84)	F(2,250) 170.74 η_p^2 0.58	< .001	acAN < recAN (p < 0.001) acAN < HC (p < 0.001) recAN < HC (p 0.007)
FT4 (pmol/L)	13.62 (1.66)	14.65 (1.88)	15.26 (1.85)	F(2,250) 21.87 η_p^2 0.15	< .001	acAN < recAN (p 0.005) acAN < HC (p < 0.001)
FT3/FT4 ratio	0.25 (0.05)	0.35 (0.05)	0.36 (0.06)	F(2,250) 109.12 η_p^2 0.47	< .001	acAN < recAN (p < 0.001) acAN < HC (p < 0.001)
TSH (mIU/L)	2.24 (1.16)	2.72 (1.14)	2.86 (1.45)	F(2,250) 3.50 η_p^2 0.03	0.032	acAN < HC (p 0.004)
Psychiatric symptom characteristics						
BDI-II total score	23.14 (11.76)	8.26 (8.11)	4.08 (4.32)	F(2,251) 135.25 η_p^2 0.52	< .001	acAN > recAN (p < 0.001) acAN > HC (p < 0.001) recAN > HC (p 0.007)
EDI-2 total score (sum)	207.18 (50.24)	163.44 (42.66)	137.88 (27.87)	F(2,247) 72.97 η_p^2 0.37	< .001	acAN > recAN (p < 0.001) acAN > HC (p < 0.001) recAN > HC (p < 0.001)
SCL-90-R GSI	.96 (.67)	.44 (.37)	.25 (.23)	F(2,250) 63.68 η_p^2 0.34	< .001	acAN > recAN (p < 0.001) acAN > HC (p < 0.001) recAN > HC (p 0.034)

Abbreviations: acAN-T1, acute anorexia nervosa participants at study time point T1 (shortly after admission); recAN, long-term weight-recovered anorexia nervosa participants; HC, healthy control participants; IQ, intelligence quotient; BMI, body mass index; BMI-SDS, body mass index standard deviation score; FT3, free triiodothyronine; FT4, free thyroxine; FT3/FT4 ratio, free triiodothyronine to free thyroxine ratio; TSH, thyroid-stimulating hormone; BDI-II, Beck Depression Inventory-II; EDI-2, Eating Disorder Inventory-2; SCL-90-R GSI, Global Severity Index of the Symptom Checklist-90-Revised; n/a, not assessed.

[a]Mean (mean value) and SD (standard deviation) for each variable are displayed separately for each study group.

[b]One-way ANOVAs were applied to detect group differences in mean regarding demographic, clinical, and psychiatric symptom variables, whereas ANCOVAs including the covariate age at date of research were employed for hormone parameters (leptin and pituitary-thyroid hormones, see SM 2.3 for potential age effects), both of which were followed up by Scheffé post-hoc tests for specifying pairwise group differences. F-values are stated for each variable (including degrees of freedom), and p-values are reported. Effect size statistics are provided as partial η^2 (η_p^2). Study data were approximately normally distributed (see SM 2.2 and Table S3 for details, see Fig. S1 for boxplots).

In acAN-T1 (n = 77), the mean (SD) age at first AN onset was 14.4 (3.2) years (assessed in n = 74) and the mean (SD) duration of the current AN episode was 16.2 (23.1) months (n = 76). The AN subtype was determined via SIAB-EX in all acAN-T1 participants: n = 68 (88.31%) were of the restrictive subtype and n = 9 (11.69%) of the binge/purge subtype, with no differences regarding clinical, hormone, and psychiatric symptom characteristics between subtypes (SM 1.1, Table S1). In recAN (n = 55), the mean (SD) age at first AN onset was 14.6 (2.1) years (assessed in n = 52) and the mean (SD) duration of recovery from the former AN episode was 55.9 (38.8) months (n = 53). The restrictive AN subtype used to be present in n = 42 (76.36%) recAN, whereas n = 13 (23.64%) used to suffer from the binge/purge subtype as indicated by evaluation with the SIAB-EX, again with no differences between former AN subtypes in relevant characteristics. See SM 2.1 and SM 2.6 for further demographic and clinical characteristics (psychiatric comorbidities, psychoactive medication) of the study sample.

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parameters and depressive symptoms would be more pronounced in acute patients with AN showing manifest low-T3 syndrome, i.e., when thyroid metabolism is clearly altered and therefore of more immediate clinical and potentially therapeutic relevance.

2. Materials and methods

2.1. Participants

The current study sample consisted of 260 female volunteers: 77 acute patients (acAN, aged 12–29 years) diagnosed with AN according to DSM-5 criteria (American Psychiatric Association, 2013), 55 individuals long-term recovered from AN (recAN, aged 16–30 years), and 128 normal-weight healthy controls (HC, aged 12–29 years).

The study was approved by the local Institutional Review Board of the TU Dresden and was carried out in accordance with the Declaration of Helsinki. All participants (and their legal guardians if underage) gave written informed consent.

AcAN were admitted to eating disorder treatment programs at a child and adolescent psychiatry or psychosomatic medicine department of a tertiary care university hospital and were recruited within 96 h after beginning behaviorally oriented inpatient renutrition programs, referred to as study time point T1 (acAN-T1). Current AN diagnosis was established through evaluation with the expert form of the Structured Interview for Anorexia and Bulimia Nervosa (SIAB-EX; Fichter and Quadflieg, 1999) adapted to meet DSM-5 criteria, which requires a BMI $<$ 10th age percentile (if younger than 15.5 years) or $<$ 17.5 kg/m^2 (if older than 15.5 years). For the longitudinal study arm, 48 acAN (aged 12–24 years at acAN-T1) were reassessed at a second study time point (acAN-T2, aged 12–24 years) after short-term weight-restoration with a minimum BMI increase of 14%.

RecAN had to: (1) be long-term weight-restored, i.e., maintain a normal BMI ($\geq$ 10th age percentile (if younger than 18 years) or $\geq$ 18.5 kg/m^2 (if older than 18 years) for a minimum of 6 months), (2) menstruate regularly, and (3) have neither binged or purged nor been engaged in any significant restrictive eating patterns for at least 6 months before the study.

HC were recruited through advertisement among middle school, high school, and university students. HC had to be eumenorrhoeic and mentally healthy, i.e., without any history of psychiatric illness and without relevant current depressive symptoms in our research interview and questionnaire-based assessment (BDI-II total scores $<$ 20 were tolerated, rated as no or subclinical depression (Hautzinger et al., 2009; Wang and Gorenstein, 2013)). HC were excluded if they showed abnormal BMI and/or eating behavior, assessed via SIAB-EX.

Additional exclusion criteria for all groups, information pertinent to exclusion criteria, AN subtypes, co-existing diagnoses, and psychoactive medication as well as possible confounders such as menstrual cycle and contraceptive medication were obtained using SIAB-EX, supplemented by our own semi-structured research interview and medical records (legends of Tables 2–3; Supplementary material (SM) 1.1, 1.2, and 2.1).

Regarding pituitary-thyroid hormone assessments, we considered any diagnosed form of a current thyroid disorder (if unrelated to the AN episode) and current thyroid medication as a-priori exclusion criteria. However, known thyroid aberrancies in the past, followed by recovery and a medication-free euthyroid state ($>$ 1 year), did not lead to exclusion (SM 1.1). In the HC group, TSH concentrations above the age-related reference range in combination with reduced FT4 occurred in n 6 participants (see SM 1.3 for reference ranges of all assessed pituitary-thyroid hormones), who were subsequently excluded from all analyses due to evidence for hitherto undiagnosed manifest hypothyroidism (Garber et al., 2012). Following these exclusions, 122 (out of 128) HC were included in the main analyses.

Table 3
Longitudinal study sample (acAN-T1-T2): demographic, clinical, leptin, pituitary-thyroid, and psychiatric symptom characteristics.

	acAN-T1[a] (n 48)	acAN-T2[a] (n 48)	Paired-samples *t*-test[b]	p
Demographic and clinical variables				
Age (years)	16.09 (2.60)	16.33 (2.59)	n/a	
BMI (kg/m^2)	14.82 (1.22)	18.78 (1.06)	|t(47)| 25.44 d 3.67	$<$ 0.001
BMI-SDS	-3.05 (1.01)	-0.75 (0.60)	|t(47)| 20.83 d 3.01	$<$ 0.001
Hormone parameters				
Leptin (µg/L)	1.79 (2.47)	12.27 (7.92)	|t(47)| 10.66 d 1.54	$<$ 0.001
Pituitary-thyroid characteristics				
FT3 (pmol/L)	3.22 (0.64)	5.33 (1.15)	|t(47)| 11.83 d 1.71	$<$ 0.001
FT4 (pmol/L)	13.61 (1.82)	13.67 (1.86)	|t(47)| 0.18 d 0.03	0.860
FT3/FT4 ratio	0.24 (0.04)	0.40 (0.10)	|t(47)| 11.11 d 1.60	$<$ 0.001
TSH (mIU/L)	2.21 (1.06)	2.48 (1.03)	|t(47)| 2.28 d 0.33	0.027
Psychiatric symptom characteristics				
BDI-II total score	22.01 (11.29)	13.34 (11.01)	|t(46)| 7.21 d 1.05	$<$ 0.001
EDI-2 total score (sum)	201.03 (47.67)	183.44 (49.58)	|t(45)| 3.27 d 0.48	0.002
SCL-90-R GSI	0.89 (0.59)	0.58 (0.47)	|t(46)| 5.70 d 0.83	$<$ 0.001

Abbreviations: acAN, acute anorexia nervosa participants at study time point T1 (shortly after admission, acAN-T1) or T2 (after short-term weight-restoration, acAN-T2); BMI, body mass index; BMI-SDS, body mass index standard deviation score; FT3, free triiodothyronine; FT4, free thyroxine; FT3/FT4 ratio, free triiodothyronine to free thyroxine ratio; TSH, thyroid-stimulating hormone; BDI-II, Beck Depression Inventory-II; EDI-2, Eating Disorder Inventory-2; SCL-90-R GSI, Global Severity Index of the Symptom Checklist-90-Revised; n/a, not assessed.
[a]Mean (mean value) and SD (standard deviation) for each variable are displayed separately for each study time point (T1 and T2).
[b]Differences in acAN between study time points T1 and T2 were tested using paired-samples *t*-tests. As test results, t-values (absolute value) are stated for each variable (including degrees of freedom), and p-values are reported. Effect sizes were estimated via Cohen's d. Study data were approximately normally distributed (see SM 2.2 and Table S3 for details, see Fig. S1 for boxplots).
The mean (SD) duration of inpatient treatment (time between study time points T1 and T2) was 86 (23) days in the longitudinal acAN sample. See SM 2.1 and SM 2.6 for clinical characteristics (psychiatric comorbidities, psychoactive medication) of the longitudinal acAN sample.

2.2. Clinical measures

To ensure comparability by age, BMI standard deviation scores (BMI-SDS), adjusted for age and gender, were calculated for statistical analyses (according to German population reference data, see SM 2.3 for details and references). Depressive symptoms were assessed via the German version of Beck Depression Inventory-II (BDI-II) as a clinically well-established measure of depressive symptom levels (Hautzinger et al., 2009; Wang and Gorenstein, 2013), ED-specific symptoms with the Eating Disorder Inventory-2 (EDI-2; Paul and Thiel, 2005), and general levels of psychopathology including psychological burdens and quality of life by means of the Global Severity Index (GSI) of the Symptom Checklist-90-Revised (SCL-90-R; Franke, 2002) (see SM 1.2 for details). IQ was estimated with short versions of the German adaptation of the Wechsler Adult Intelligence Scale (WIE; von Aster et al., 2006) or the Wechsler Intelligence Scale for Children (HAWIK-IV; Petermann and Petermann, 2011), the latter for study participants aged 15 years or younger. Demographic and clinical study data were collected and managed using a secure, web-based electronic data capture tool (REDCap; Harris et al., 2009).

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Table 4
Cross-sectional study sample (acAN-T1, recAN): table of correlations (Spearman).

Correlated variables: Pituitary-thyroid and psychiatric symptoms		acAN-T1 (n = 77)			acAN-T1-low-FT3 subgroup (n = 63)			recAN (n = 55)		
		BDI-II total	EDI-II total[a]	SCL-90-R GSI	BDI-II total	EDI-2 total[b]	SCL-90-R GSI	BDI-II total	EDI-2 total[c]	SCL-90-R GSI[d]
FT3	$r_{S,\ partial\ (age)}$	**-0.25**	-0.14	-0.18	-0.39 *	-0.32 *	-0.32 *	0.11	0.06	-0.02
		(p = 0.030)	(p = 0.225)	(p = 0.129)	(p = 0.002)	(p = 0.011)	(p = 0.010)	(p = 0.439)	(p = 0.689)	(p = 0.897)
FT4	$r_{S,\ partial\ (age)}$	-0.03	0.09	-0.08	-0.09	0.02	-0.16	0.01	0.00	-0.02
		(p = 0.772)	(p = 0.463)	(p = 0.509)	(p = 0.488)	(p = 0.904)	(p = 0.203)	(p = 0.935)	(p = 0.996)	(p = 0.903)
FT3/FT4 ratio	$r_{S,\ partial\ (age)}$	**-0.23**	-0.17	-0.11	-0.33 *	-0.33 *	-0.21	0.07	0.03	-0.07
		(p = 0.046)	(p = 0.147)	(p = 0.338)	(p = 0.009)	(p = 0.010)	(p = 0.108)	(p = 0.637)	(p = 0.806)	(p = 0.641)
TSH	$r_{S,\ partial\ (age)}$	0.15	0.07	0.09	0.17	0.10	0.13	-0.14	-0.03	-0.16
		(p = 0.208)	(p = 0.559)	(p = 0.427)	(p = 0.181)	(p = 0.434)	(p = 0.312)	(p = 0.319)	(p = 0.828)	(p = 0.244)

Abbreviations: acAN-T1, acute anorexia nervosa participants at study time point T1 (shortly after admission); recAN, long-term weight-recovered anorexia nervosa participants; FT3, free triiodothyronine in pmol/L; low-FT3, plasma FT3 concentrations below age-related reference range (low-T3 syndrome); FT4, free thyroxine in pmol/L; FT3/FT4 ratio, free triiodothyronine to free thyroxine ratio; TSH, thyroid-stimulating hormone in mIU/L; BDI-II, Beck Depression Inventory-II; EDI-2, Eating Disorder Inventory-2; SCL-90-R GSI, Global Severity Index of the Symptom Checklist-90-Revised.
Spearman correlation coefficients between pituitary-thyroid hormone parameters (FT3, FT4, FT3/FT4 ratio, TSH) and psychiatric symptom characteristics (BDI-II total, EDI-2 total (sum), SCL-90-R GSI) are presented for cross-sectional study groups including the acAN-T1-low-FT3 subgroup (see SM 2.5, Table S7 for correlations in HC). Owing to potential age effects (SM 2.3), partial correlations with participants' age as the covariate ($r_{S,\ partial\ (age)}$) were computed (see SM 2.3 and SM 2.5, Table S8 for added BMI-SDS adjustment in acAN-T1 including the acAN-T1-low-FT3 subgroup). Due to missing EDI-2 in 2/77 acAN-T1[a,b], missing EDI-2 in 1/55 recAN[c] and missing SCL-90-R GSI in 1/55 recAN[d], the following correlations were performed with a reduced number of participants: [a] with n = 75 and [b] with n = 62 acAN-T1, [c] with n = 54 and [d] with n = 54 recAN. Study group-wise correction of p-values for multiple pairwise comparisons was applied according to the Benjamini-Hochberg false discovery rate (FDR) across all correlation tests, except for correlations regarding our main hypothesis (**BDI-II**, in **bold**) in the acAN-T1 group (n = 77). Raw p-values for all assessed correlations are reported in (). Significant ($p < 0.05$) hypothesis-driven correlations involving BDI-II are shown in **bold**, whereas significant FDR-corrected (p adjusted <0.05) correlations are designated as *.
The supplementary exclusion of acAN-T1 (n = 4) and recAN (n = 3) on antidepressant medication (selective serotonin reuptake inhibitors) did not remarkably change the above-reported correlation findings (SM 2.6).

2.3. Procedure

Peripheral venous blood was collected into vacutainer tubes containing EDTA anticoagulant between 7 and 9 a.m. after an overnight fasting period. AcAN-T1 were assessed within 96 h after initiating inpatient treatment (also between 7 and 9 a.m. after an overnight fast) to avoid early effects of renutrition. To yield blood plasma, the samples were immediately processed as follows: addition of the serine protease inhibitor aprotinin (activity 270 KIU/ml whole blood), centrifugation (at ϑ 5 °C and a 2500 x g for 15 min), aliquotation, storage at ϑ -80 °C until further analysis.

Plasma concentrations of the pituitary-thyroid hormones FT3, FT4, and TSH in all study blood samples were measured in one separate session at the same laboratory using commercially available electrochemiluminescence immunoassays (ECLIA) according to the manufacturer's instructions (Roche Diagnostics GmbH, Mannheim, Germany). The ECLIAs have been demonstrated to quantify pituitary-thyroid hormone concentrations at high sensitivity and precision with intra- and inter-assay variation coefficients of < 5% (SM 1.3, Table S2). The manufacturer's age-related reference intervals were regarded for the clinical interpretation of pituitary-thyroid function in adolescent and adult study participants (SM 1.3) and the lower limit of reference range for FT3 was used to define low-T3 syndrome in acAN-T1 (acAN-T1-low-FT3 subgroup). To indicate the conversion rate from FT4 into FT3, likely to represent deiodinase (D2) activity (Yu et al., 2018), a ratio of both thyroid hormone concentrations (FT3/FT4 ratio) was calculated as an indirect conversion index.

Plasma leptin was assessed as an indicator variable of nutritional status and the severity of neuroendocrine alterations (Focker et al., 2011; Misra et al., 2005) in patients with AN (SM 1.3 and SM 2.5, Table S8). Leptin concentrations were measured using a commercially available enzyme-linked immunosorbent assay (ELISA; BioVendor Research and Diagnostic Products, Brno/Czech Republic) with intra- and inter-assay variation coefficients of < 10% (SM 1.3, Table S2).

2.4. Statistical analyses

One-way analyses of variance (ANOVAs) and Scheffé post-hoc tests were employed for cross-sectional comparisons of acAN-T1, recAN, and HC regarding demographic, clinical, and psychiatric symptom variables (Table 2). To account for potential age effects on hormone concentrations (SM 2.3), age-adjusted analyses of covariance (ANCOVAs) were performed for leptin and pituitary-thyroid parameters, followed by Scheffé pairwise comparisons. Paired-samples *t*-tests were conducted in the longitudinal acAN sample (Table 3).

Relationships between pituitary-thyroid hormone parameters and BDI-II total scores were assessed via Spearman rank-order correlations within individual cross-sectionally studied groups (acAN-T1, acAN-T1-low-FT3 subgroup, and recAN, Table 4) as well as longitudinally over the course of short-term weight-restoration of acAN (acAN-T1 – acAN-T2) via change scores (Δ T2 - T1) of pituitary-thyroid hormone parameters (Table 5). Besides our hypothesis-driven approach for depressive symptoms, we further explored correlations of pituitary-thyroid hormone parameters with ED (via EDI-2) and general psychiatric (via SCL-90-R) symptom scores. Cross-sectional correlations were adjusted for age at date of research as the covariate ($r_{S,\ partial\ (age)}$). Study group-wise multiple testing correction of p-values according to the Benjamini-Hochberg false discovery rate (FDR; Benjamini and Hochberg, 1995) was applied across all correlation tests except for correlations regarding our main hypothesis (BDI-II) in acAN-T1 (Table 4) and in the longitudinal analysis (Table 5). Please consult SM Fig. S2 for a flowchart summary of statistical analyses.

Previous studies with smaller sample sizes found significant (α 0.05) group differences in pituitary-thyroid hormone parameters (example: FT3 concentrations) between patients with AN and controls which correspond to large effect sizes, e.g., Cohen's d 4.91 (10 AN, 18

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Table 5
Longitudinal study sample (acAN-T1-T2): table of correlations (Spearman).

Correlated variables: Pituitary-thyroid and psychiatric symptoms		Longitudinal acAN sample (n 47)		
		BDI-II total at T2	EDI-2 total at T2	SCL-90-R GSI at T2
ΔFT3	r_S	-0.16	-0.13	-0.30
		(p 0.297)	(p 0.368)	(p 0.043)
ΔFT4	r_S	0.27	0.27	0.31
		(p 0.070)	(p 0.070)	(p 0.032)
ΔFT3/FT4 ratio	r_S	**-0.31**	-0.29	-0.46 *
		(p 0.032)	(p 0.045)	(p 0.001)
ΔTSH	r_S	0.00	-0.07	0.08
		(p 0.978)	(p 0.657)	(p 0.607)

Abbreviations: acAN, acute anorexia nervosa participants at study time point T1 (shortly after admission, acAN-T1) or T2 (after short-term weight-restoration, acAN-T2); Δ, indicator of change T2 - T1; FT3, free triiodothyronine in pmol/L; FT4, free thyroxine in pmol/L; FT3/FT4 ratio, free triiodothyronine to free thyroxine ratio; TSH, thyroid-stimulating hormone in mIU/L; BDI-II, Beck Depression Inventory-II; EDI-2, Eating Disorder Inventory-2; SCL-90-R GSI, Global Severity Index of the Symptom Checklist-90-Revised.
Spearman correlation coefficients (r_S) between change scores of pituitary-thyroid hormone parameters across study time points T1 and T2 (T2 - T1) (ΔFT3, ΔFT4, ΔFT3/FT4 ratio, ΔTSH) and T2-symptom characteristics (BDI-II total at T2, EDI-2 total (sum) at T2, SCL-90-R GSI at T2) are presented in the longitudinal acAN sample (see SM 2.3 and SM 2.5, Table S8 for added ΔBMI-SDS adjustment). Due to missing symptom data in 1 acAN-T2, longitudinal correlations were run in n = 47/48 acAN. Correction of p-values for multiple pairwise comparisons was applied according to the Benjamini-Hochberg false discovery rate (FDR) across all correlation tests, except for correlations regarding our main hypothesis (**BDI-II**, in **bold**). Raw p-values for all assessed correlations are reported in (). Significant ($p < 0.05$) hypothesis-driven correlations involving BDI-II are shown in **bold**, whereas significant FDR-corrected (p adjusted <0.05) correlations are designated as * .
The supplementary exclusion of acAN-T1-T2 (n = 2) on antidepressant medication (selective serotonin reuptake inhibitor and mirtazapine) did not remarkably change the above-reported correlation findings (SM 2.6). Exploratively (not shown), absolute pituitary-thyroid hormone parameters at T2 (FT3, FT4, FT3/FT4 ratio, and TSH at T2) were correlated with T2-symptom characteristics which revealed persisting associations with psychiatric symptoms for FT3/FT4 ratio at acAN-T2.

controls; Buehren et al., 2011), d 1.79 (28 AN, 49 controls; Onur et al., 2005), and d 0.85 (61 AN, 52 controls; Śmiarowska et al., 2014). Therefore, we assumed that our study including n_{total} 254 participants in the main analyses would be sufficiently powered.

IBM SPSS statistical software package for Windows version 27.0 (IBM Corp., Armonk, NY) was used for statistical analyses, supplemented by R version 4.0.3 (R Core Team, 2020) for ANCOVAs followed by Scheffé post-hoc and for partial correlation procedures, the latter based on residualization via multiple regression analysis (see SM 2.4, Fig. S2 for details on applied R packages).

3. Results

3.1. Sample characteristics

Demographics and clinical characteristics are displayed in Table 2 for the cross-sectional and in Table 3 for the longitudinal study sample. As expected, acAN-T1 had significantly lower BMI and BMI-SDS than recAN and HC and, correspondingly, substantially lower plasma leptin concentrations. Likewise, acAN-T1 presented with higher symptom scores (BDI-II and EDI-2 total scores, SCL-90-R GSI). RecAN had slightly lower BMI-SDS and leptin concentrations compared to HC and showed some residual psychopathology on the aforementioned scales.

During inpatient treatment, BMI in the acAN group increased on average by 27.30% (SD: 9.17%, range: 14.06 – 49.65%), which was accompanied by the normalization of leptin concentration (acAN-T2 vs. HC, see Table S6) and further by noteworthy improvements in depressive, ED, and general psychiatric symptom levels. However, BMI-SDS at acAN-T2 was still below the age- and sex-appropriate average (Table 3).

3.2. Pituitary-thyroid characteristics

For all pituitary-thyroid hormone parameters in our cross-sectional analyses, significant overall group differences across acAN-T1, recAN, and HC were detectable according to age-adjusted omnibus ANCOVAs (Table 2). Scheffé post-hoc tests yielded lower FT3 concentrations in acAN-T1 than in recAN and HC. Besides, lower FT3 concentrations occurred in recAN in comparison to HC (Table 2, Fig. S3(A)). Out of 77 acAN-T1, n 63 (81.82%) had plasma FT3 concentrations below age-related reference range (SM 1.3) and, thus, belonged to the acAN-T1-low-FT3 subgroup with clinically manifest low-T3 syndrome (see Table S4 for statistical details on this subgroup). In contrast, n 0 of the 55 recAN and only n 1 of the 122 HC had FT3 concentrations below reference range. Plasma FT4 concentrations were lower in acAN-T1 than in recAN and HC, with no difference between recAN and HC. However, only n 20 of 77 acAN-T1 (25.97%) had FT4 concentrations below reference range. Regarding the conversion ratio FT3/FT4, acAN-T1 presented with a significantly lower mean ratio than recAN and HC, whereas recAN did not differ from HC (Table 2, Fig. S3(C)). Further, post-hoc testing revealed reduced TSH concentrations in acAN-T1 compared to HC (Table 2, Fig. S3(D)). When HC with latent thyroid hypofunction (slightly elevated TSH but normal FT4) and/or subclinical BDI-II total scores were excluded as part of a supplementary analysis, the above-reported between-group differences remained significant except for TSH (see Table S5 for details, adjusted HC group with n 97 HC).

After short-term weight-restoration of longitudinally studied acAN, FT3 concentrations were significantly increased (Table 3, Fig. S3(E)), accompanied by a decline in the prevalence of low-T3 syndrome (n 42 of 48 acAN-T1 (87.50%) vs. n 2 of 48 acAN-T2 (4.17%)), whereas FT4 concentrations did not change across study time points. The conversion ratio FT3/FT4 and also TSH concentrations were markedly higher at acAN-T2 than acAN-T1 (Table 3, Fig. S3(G/H)). Compared to HC, acAN-T2 still had lower FT3 and FT4 concentrations along with several other clinical alterations (e.g., higher psychiatric symptom scores, Table S6).

3.3. Associations of pituitary-thyroid characteristics with psychiatric symptoms

Lower FT3 concentrations and FT3/FT4 ratios were associated with more severe depressive symptoms (i.e., higher BDI-II total scores) in acAN-T1 according to age-adjusted correlations (Table 4, Fig. S4(A/C)). This was especially pronounced in the acAN-T1-low-FT3 subgroup (Table 4, Fig. S4(B/D)). Exploratory analyses demonstrated that, exclusively in the acAN-T1-low-FT3 subgroup (i.e., not in the entire acAN-T1 group), FT3 concentrations and FT3/FT4 ratios were inversely correlated with EDI-2 total scores and FT3 concentrations were further

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inversely correlated with SCL-90-R GSI. However, significant correlations between FT4 or TSH concentrations and any of the psychiatric symptom scores could not be detected in acAN-T1 (Table 4). Furthermore, no significant associations between pituitary-thyroid hormone parameters and psychiatric symptoms emerged for recAN (Table 4, see Table S7 for correlations in HC which were likewise non-significant).

Regarding the longitudinally studied acAN sample, change scores of the conversion ratio FT3/FT4 correlated inversely with depressive symptoms at acAN-T2 (Table 5, Fig. S4(E)). Exploratory analyses revealed that a larger positive change score of FT3/FT4 ratio was further associated with reduced general psychopathology at acAN-T2 as assessed via SCL-90-R GSI. Following FDR-correction, no significant correlations occurred for the remaining change scores of pituitary-thyroid hormone parameters with any of the assessed psychiatric symptom measures at acAN-T2 in the longitudinal study arm.

According to a supplementary partial correlation analysis controlling for the variation in BMI-SDS (in cross-sectionally studied acAN-T1 including the acAN-T1-low-FT3 subgroup) or ΔBMI-SDS (in longitudinally studied acAN), most correlation findings remained significant, i.e., they were not solely accounted for by the severity of acute underweight or the amount of BMI-SDS increase over the course of short-term weight-restoration (Table S8).

4. Discussion

In the current study, we analyzed alterations of pituitary-thyroid hormones in relation to psychiatric symptom levels, in particular depressive symptoms, in a relatively large sample of young females with acutely underweight AN and again after short-term weight-restoration as well as in long-term weight-recovered individuals and healthy controls. In acAN-T1, we found a significant reduction in all measured pituitary-thyroid hormones and, further, in the computed conversion ratio FT3/FT4 indirectly representing the rate of peripheral FT4 deiodination into bioactive FT3. Short-term weight-restoration was accompanied by partial recovery of pituitary-thyroid function. Cross-sectional correlation analysis revealed significant inverse associations of FT3 concentrations and, respectively, conversion ratios FT3/FT4 with depressive symptoms in acAN-T1, which was particularly prominent in the subgroup with low-T3 syndrome (occurred in circa 82% of acAN-T1). A greater increase in FT3/FT4 ratio during weight gain was linked to a reduction in depressive and general psychiatric symptoms at follow-up. This study is the first in AN to place thyroid hormone conversion impairment in acute AN (via FT3/FT4 ratio) into the context of psychiatric symptom levels. Our findings suggest a potential modulatory role of altered pituitary-thyroid function (FT3, FT3/FT4 ratio) on depressed mood in AN.

Regarding HPT axis alterations in AN, the current study confirms findings of previous studies that have detected substantially decreased T3 or FT3 concentrations in the acutely underweight state of AN with a rapid increase after initiating weight-restoration treatment (Estour et al., 2010; Onur et al., 2005; Reinehr et al., 2008; ŚmIarowska et al., 2014; Swenne et al., 2009). In support of the key role of FT3 alterations in AN, cross-sectional group differences for FT3 and its longitudinal change in acAN had the largest effect sizes among the assessed pituitary-thyroid hormone parameters in our study, closely followed by FT3/FT4 ratio (Tables 2–3). So far, evidence for residual HPT axis alterations in former AN, i.e., after long-term weight-recovery, is sparse. Significantly increased FT3 concentrations compared to the acutely underweight state were previously reported in a small AN sample (n 9) at reassessment after gaining > 5% weight over the course of one year (Reinehr et al., 2008). Interestingly, in our recAN group, slightly lower FT3 concentrations than in HC persisted after on average 5 years of weight maintenance, although low-T3 syndrome did not occur. Speculative explanations for this preliminary finding might be either persisting BMI-SDS differences compared to HC or a special diet likely to affect FT3 concentrations (low carbohydrate/high protein (Azizi, 1978)).

Regarding the examined associations between pituitary-thyroid hormone parameters and psychiatric symptoms, we found that, in the acute state of AN, lower FT3 concentrations were associated with higher depressive symptom levels, especially in patients with low-T3 syndrome. This finding points toward state-related associations between thyroid and depressive symptom characteristics in AN which seem to have greater relevance under clinically manifest thyroid dysfunction, in line with our main hypothesis. Within this framework, the co-occurrence of lower FT4 and also leptin concentrations in patients with AN and low-T3 syndrome (compared to their normal-FT3 counterparts, Table S4) highlights their more severe neuroendocrine impairment.

There is only one study in AN to date (n 10; Buehren et al., 2011) that has provided preliminary evidence for significant inverse relationships between low FT3 concentrations and depressive symptoms in line with our cross-sectional findings in acAN-T1. Brambilla et al. (2006) likewise reported inverse relationships between FT3 concentrations and depressive symptoms but only in patients with ED not-otherwise-specified (EDNOS, n 8) and not in patients with typical AN. On the contrary, two pilot studies did not observe any cross-sectional or longitudinal associations between T3 concentrations and either dysphoric or psychovegetative aspects of depressive symptoms in AN (Laessle et al., 1988; Pahl et al., 1985). Moreover, there are some discrepancies between the current and previous studies regarding single pituitary-thyroid hormones in relation to psychopathology: While FT4 and TSH concentrations were less affected by acute AN than FT3 in our study and unrelated to depressive symptoms, a study on > 200 patients with different EDs proposed higher odds of co-existing depression for patients with lower FT4 concentrations, whereas findings for T3 and TSH were non-significant (Swenne and Rosling, 2010). Differences to our results might be caused by Swenne and Rosling's approach to analyze adolescent girls diagnosed with bulimia nervosa and EDNOS in a combined sample together with patients with AN. There is evidence for differing neuroendocrine, including HPT axis profiles between ED diagnoses (Warren, 2011). Besides, in comparison to the acAN-T1 group in our study, BMI, T3, and FT4 levels were less severely affected in the ED patients of Swenne and Rosling's study. Another study on AN (Brambilla et al., 2006) stated heterogeneous (partly positive/negative) associations of FT3, FT4, and TSH concentrations with several subscales of ED-specific and general psychiatric symptom measures differing across AN subtypes (apart from their findings for depressive symptoms as outlined above). Owing to congruent hormone and psychiatric symptom characteristics in restrictive and binge/purge acAN in our study (Table S1), we could not distinguish between AN subtypes. In contrast to Brambilla et al. (2006), our exploratory approach covering ED and general psychiatric symptoms revealed negative correlations with FT3 concentrations and FT3/FT4 ratios in acAN-T1 affected by low-T3 syndrome. This may be interpreted in addition to our main finding for depressive symptoms, suggesting that associations between altered pituitary-thyroid function and psychiatric symptoms extend to diverse facets of psychopathology in AN. Some previous studies have also examined T3 or FT3 alterations in the light of global AN-related symptoms and neuropsychiatric aspects like visuospatial performance, learning and memory functions in patients with AN reporting partly significant findings of inverse associations (Pahl et al., 1985) but otherwise non-significant results (Andrés-Perpina et al., 2011; Buehren et al., 2011; ŚmIarowska et al., 2014). Finally, heterogeneous findings of studies on associations between pituitary-thyroid hormones and psychopathology in AN might be explained by differing age ranges and duration of illness of participants, T3 vs. FT3 measurements, applied hormone assays, and fasting vs. non-fasting time of blood sampling due to circadian rhythms of HPT axis hormones (Table 1; Brambilla et al., 2006; Buehren et al., 2011; Laessle et al., 1988; Pahl et al., 1985; Støving et al., 2001; Swenne et al., 2009; Swenne and Rosling, 2010).

In acAN-T1, correlational relationships between FT3/FT4 ratios and depressive symptoms were found to be similar to the ones including FT3

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concentrations. We therefore suspect that low FT3/FT4 ratios might be of a psychiatric relevance comparable to FT3 alterations in acute AN. Further, FT3/FT4 ratio may serve as a surrogate marker of low-T3 syndrome in acute AN, mirroring the severely reduced FT3 availability relative to less affected FT4 concentrations, which replicates a recent study in AN and critically ill patients where a cutoff for FT3/FT4 ratio was introduced to flag peripheral low-T3 syndrome (Nomura et al., 2017).

During short-term weight-restoration in longitudinally studied patients with AN, FT3/FT4 ratio change score was the only pituitary-thyroid hormone parameter significantly linked to post-treatment severity of depressive and general psychiatric symptoms. Therefore, we speculate that longitudinally increasing FT4 conversion into FT3 could correspond more closely to metabolic recovery with possibly favorable impacts on mood than the absolute rise in FT3 concentration. In support of this interpretation, but from the area of euthyroid sick syndrome research, FT3/FT4 ratio has been identified as an independent predictor of frailty and survival in hospitalized geriatric patients and of 1-year mortality after myocardial infarction (Pasqualetti et al., 2018; Yu et al., 2018). In the field of AN research, literature is confined to one longitudinal study reporting non-significant relationships between thyroid function (only FT3 assessed) and depressive symptoms after 4-month inpatient treatment (Buehren et al., 2011). Nonetheless, we wish to acknowledge the relative character of FT3/FT4 ratio and its shortcoming of not entirely representing thyroid recovery during weight-restoration. Exemplarily, we observed longitudinally increasing and at acAN-T2 normalized FT3/FT4 ratios but no longitudinal change in FT4, the latter going in line with previous research (Leslie et al., 1978; Onur et al., 2005; Tamai et al., 1986). Thyroid gland atrophy in AN, speculated to be involved in a "vicious circle" maintaining co-existing psychopathology (Støving et al., 2001), might underlie the post-treatment thyroid hormone aberrancies as well as the significant longitudinal associations between thyroid and psychiatric symptom characteristics detected by our study. Likewise, a recent meta-analysis demonstrated persisting FT3 differences between acute patients with AN post-treatment and controls (Hübel et al., 2019).

To our knowledge, the present study is the first in AN to also investigate relationships between pituitary-thyroid hormone parameters and psychiatric symptoms in individuals with former AN after long-term weight-recovery. Since noteworthy relationships were absent in recAN and, thus, mostly in agreement with our non-significant correlation results in HC, we conclude that associations between thyroid and psychiatric symptom characteristics might only be relevant in AN when thyroid metabolism is acutely affected (i.e., in acAN).

With respect to potential clinical implications of the present study for acute AN, we found that the degree of underweight (BMI-SDS) varying within acAN-T1 or the amount of weight gain in the longitudinal study arm did not drive correlation findings for FT3 and FT3/FT4 ratio. Therefore, our main findings indicate rather direct relationships of FT3 and FT3/FT4 ratio alterations to depressive symptoms that might not dissolve solely through weight-rehabilitation. Given that antidepressant medication taken by a handful of acAN and recAN participants included in our study (selective serotonin reuptake inhibitors and mirtazapine, SM 2.1) might also influence pituitary-thyroid hormone concentrations (Hage and Azar, 2012), we indirectly demonstrated by excluding the antidepressant-treated participants in a supplementary analysis (SM 2.6) that there were no significant confounding effects of antidepressant intake on the associations between pituitary-thyroid hormones and depressive symptoms in our study. Consequently, the prevalence and degree of low-T3 syndrome and thyroid hormone conversion impairment might contribute to the modulation of the severity of depressive symptoms and also ED-specific and general psychiatric symptoms in AN. This seems to go beyond the acutely underweight state and persist into short-term weight-restoration.

Our findings may be considered in the light of high levels of depressive symptoms in AN, the psychiatric disorder with the highest standardized mortality rate and often poor long-term psychiatric outcome (Arcelus et al., 2011; Steinhausen, 2002), and against the background of the current search for psychopharmacological targets as part of multimodal treatment approaches for AN (Schaumberg et al., 2017). Hence, we would like to stimulate a very cautious discussion on potential benefits of experimental low-dose thyroid hormone supplementation for severely affected patients with AN, presenting with manifest low-T3 syndrome and suffering from serious depressive or suicidal symptoms. Mood stabilization, compliance with renutrition programs due to possibly increasing appetite (Mullur et al., 2014), and improving responsiveness to antidepressants might be speculative therapeutic aims for temporary thyroid hormone supplementation in acute AN (e.g., during inpatient treatment), preferably via combination therapy with LT4 and LT3 to bypass the AN-related conversion deficiency. The evident efficacy of adjunctive thyroid hormone usage in other fields of psychiatry like depressive disorders (Bauer and Whybrow, 2021) lends some support to this speculation. However, as an important counterargument, pituitary-thyroid hormone alterations are widely considered as an adaptive and/or protective response to acute weight loss. Furthermore, thyroid hormone supplementation has not been studied in AN yet and current clinical review articles recommend against it because of potential adverse effects on weight gain, arrhythmias (Lawson and Klibanski, 2008; Lee and Farwell, 2016), or the realistic risk of abuse in AN shown by an early case report (Woodside et al., 1991).

4.1. *Limitations*

The following limitations to the current study should be taken into consideration: First, our pituitary-thyroid hormone assessments offer insight into peripheral metabolism, whereas the central availability and conversion of FT3 and FT4, mainly regulated via the blood-brain barrier passage of FT4 and its brain-specific deiodination (Hage and Azar, 2012; Swenne and Rosling, 2010), would be of particular interest for neuropsychiatric investigations. Second, we wish to point out the complex clinical appearance of acute AN with multiple ceiling or mediating effects on neuroendocrine and psychiatric characteristics (e.g., inflammatory cytokines (Lawson and Klibanski, 2008) or psychosocial stress) that we cannot sufficiently account for. Third, a longitudinal assessment of the HC group would have been needed to capture natural variations over time and in distinction to the observations in longitudinally studied patients with AN. Fourth, more frequent assessments during weight-restoration would have been useful to fully understand the longitudinal course of associations between pituitary-thyroid hormone parameters and psychiatric symptoms and to unveil potential predictors of outcome. Finally, the longitudinal cohort of patients with AN was not followed up long-term so that findings cannot be generalized regarding, exemplarily, chronic courses of AN. The observed non-significant associations in the independent recAN group may reflect a higher likelihood of recovery from AN in these individuals.

5. Conclusions

This study provides novel evidence for a state-related inverse association between HPT axis alterations, predominantly low-T3 syndrome as well as low thyroid hormone conversion, and depressive symptoms in acutely underweight and short-term weight-restored patients with AN. Our findings might encourage clinicians to review routine thyroid assessments at ED units in the context of a patient's affective condition. For monitoring thyroid function along with depressive symptoms in AN, conversion ratios FT3/FT4 might be of hitherto unrecognized clinical value. In order to understand the mechanisms underlying our findings, such as thyroid hormone actions in the brain and plausible impacts on neurotransmitter systems in AN, and to evaluate therapeutic perspectives, further research efforts are vital: For instance, studies using animal models of AN (Méquinion et al., 2015), neuroimaging studies, or,

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possibly, randomized controlled clinical trials with low thyroid hormone doses in severely depressed patients with AN might be promising.

CRediT authorship contribution statement

Marie-Louis Wronski: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft. **Friederike I. Tam:** Methodology, Investigation, Writing – review & editing, Supervision. **Maria Seidel:** Methodology, Data curation, Writing – review & editing, Supervision. **Peter Mirtschink:** Methodology, Resources, Writing – review & editing. **David M. Poitz:** Methodology, Visualization, Resources, Writing – review & editing. **Klaas Bahnsen:** Investigation, Software, Writing – review & editing. **Jonas L. Steinhauser:** Investigation, Writing – review & editing. **Michael Bauer:** Resources, Funding acquisition, Writing – review & editing. **Veit Roessner:** Resources, Funding acquisition, Writing – review & editing. **Stefan Ehrlich:** Conceptualization, Methodology, Validation, Resources, Funding acquisition, Writing – review & editing, Supervision, Project administration.

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Declaration of Competing Interest

V.R. has received payment for consulting and writing activities from Lilly, Novartis, and Shire Pharmaceuticals/Takeda, lecture honoraria from Lilly, Novartis, Shire Pharmaceuticals/Takeda, and Medice Pharma, and support for research from Novartis and Shire Pharmaceuticals/Takeda. V.R. has carried out (and is currently carrying out) clinical trials in cooperation with the Novartis, Shire Pharmaceuticals/Takeda, and Otsuka companies. V.R. has no financial relationship with the organizations that sponsored the research. F.T. has received a research grant from the “Marga und Walter Boll-Stiftung”. M.W., M.S., P. M., D.M.P., K.B., J.S., M.B., and S.E. declare no biomedical financial interests or potential conflicts of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psyneuen.2021.105630.

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