The impact of total body fat mass, and of its distribution in the trunk, on thyroid hormone levels after complete weight restoration, with or without recovery of menses, in adolescents with anorexia nervosa

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ABSTRACT

Background and purpose: To determine the impact of total body fat mass, and of its distribution in the trunk, on thyroid hormone levels after complete weight restoration, with or without recovery of menses, in adolescents with anorexia nervosa (AN).

Methods: Prospective study of 60 adolescents with AN and amenorrhea. Anthropometrics, body composition and hormonal studies were obtained at the beginning of the study and at complete weight restoration, whether (Group A) or not (Group B) menses were recovered.

Results: In both groups, free triiodothyronine (FT3), free thyroxine (FT4) and thyroid-stimulating hormone (TSH) levels were statistically significantly higher ($p<0.001$) at the end of the study compared with the time of first attendance. At weight restoration, a statistically significantly positive correlation was found between total body fat mass (Kg and %), trunk fat mass (Kg and %) and FT3 and FT4 levels in both Group A and Group B adolescents. At the same time point, trunk/extremities fat *ratio* was found to be statistically significantly positively correlated with FT3 ($r=0.586$, $p<0.001$) and FT4 ($r=0.512$, $p<0.01$), but not with TSH, in girls who recovered their menses, in contrast with adolescents who remained amenorrheic.

Conclusions: Total body fat mass and its distribution in the trunk were found to be statistically significantly positively correlated with FT3 and FT4 in adolescents with AN who completed restored their weight and recovered their menses.

KEYWORDS

Anorexia nervosa, body composition, thyroid dysfunction, adolescence, amenorrhea.

Introduction

Anorexia nervosa (AN) is a disease primarily affecting girls: 95% of cases are female adolescents [1]. Its prevalence ranges from 0.5% to 1%, although some studies report higher levels [2,3]. The new DSM-V criteria for the diagnosis of AN were recently published. The main difference in these new criteria is the deletion of criterion D, requiring amenorrhea for diagnosis of the disease [4].

It is well known that starvation affects the hypothalamic-pituitary-thyroid axis, leading to decreased plasma free triiodothyronine (FT3) concentration, along with decreased plasma free thyroxine (FT4) and increased plasma reverse triiodothyronine (rT3) levels; thyroid-stimulating hormone (TSH) concentration is usually normal, although decreased levels of this hormone have been reported [5,6]. This picture represents what is known as "euthyroid sick syndrome" [7,8]. The low levels of circulating T3 have the effect of reducing energy expenditure and muscle protein catabolism. Weight gain leads to elevation of FT3 and decrease of rT3 levels.

Article history
Received 28 Mar 2019 - Accepted 4 Jul 2019

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Interestingly, AN adolescents present many clinical features of hypothyroidism (bradycardia, hypothermia, delayed ankle reflexes), which lead to energy conservation. However, thyroid hormone treatment is inappropriate, leading to further weight and muscle mass loss [9]. Notably, resting energy expenditure (REE) has also been found to be significantly lower in adolescents with AN compared with healthy controls, seemingly representing an adaptive mechanism to chronic starvation [10,11]. Furthermore, the finding that re-feeding and weight restoration in girls with AN leads to increase of REE, related to changes occurring in T3 levels, highlights the role of T3 in the

regulation of energy hemostasis [12]. Finally, starvation in AN in combination with lowered metabolic rate leads to increased plasma cortisol levels (as starvation stimulates gluconeogenesis and decreases peripheral glucose utilization), as well as decreased gonadotropin levels, which are also expressions of adaptive mechanisms.

Materials and Methods

A hundred and ninety-one female adolescents presented at our Division [13] with secondary amenorrhea and a body mass index (BMI) <5th percentile for age (based on BMI charts for Greek females). Of these, 94 fulfilled the DSM-IV diagnostic criteria for AN and 60 were finally included in our prospective study. All girls were diagnosed with restricting-type AN; 34 adolescents were excluded from the study for the following reasons: a past history of hypothyroidism (n=10), smoking (n=12), and use of hormone replacement therapy (n=12).

At first attendance, a thorough medical history was recorded, including any past medical history, family history, medication or surgeries. Demographics were also taken into consideration. All girls were provided with menstrual calendar in which age at menarche, minimum and maximum cycle length, menstrual irregularities and time of secondary amenorrhea were recorded. Finally, the adolescents also provided information on past use of hormonal medication, age at diagnosis of AN, BMI at diagnosis of AN, physical activity (measured in hours/week), nutritional habits and life stress events. All the girls included in the study were non-smokers, with no medical history of any other endocrine disorder, while hormonal contraceptive use had been stopped for a minimum of 6 months prior to the time of first attendance. Informed consent to participate in the study was obtained from the adolescents and/or their parents, if needed, while the protocol for the research project, which conforms with the provisions of the Declaration of Helsinki, was approved by the ethics committee of our institution.

All adolescents underwent routine pedogynecological examination, with assessment by Tanner stages of breast and pubic hair development, while BMI (calculated by dividing weight in kilograms by the square of height in meters), waist circumference, hip circumference and waist circumference/hip circumference *ratio* were calculated. Additionally, blood samples were obtained between 8:00 and 10:00 AM after overnight fasting, always at the same time in the morning for the determination of endocrine profile. After coagulation, the samples were centrifuged and the serum was stored at –20°C until further processing. 17b-estradiol, prolactin, insulin, FT3, FT4 and TSH were measured by chemiluminescent immune assays, while follicle-stimulating hormone, luteinizing hormone and leptin were measured by immunoradiometric assay and cortisol was analyzed by radioimmunoassay. Pelvic ultrasonography was performed in all adolescents to exclude any pathology of the uterus and/or the ovaries, while full body composition analyses were performed based on (dual energy X-ray absorptiometry) DXA scans (GE Lunar Prodigy apparatus enCore, 2008, USA); these analyses included measurement of total body fat mass (Kg and % of total body tissue), trunk fat mass (Kg and % of total trunk tissue) and free fat mass (Kg and % of total body tissue). Finally, trunk/extremity (right arm and left arm + right leg and left leg) fat *ratio* was calculated.

The abovementioned examinations were performed in all adolescents at first attendance. Subsequently, the girls followed a nutritional rehabilitation program under the supervision of a nutritionist until complete weight restoration. This was set at BMI>10th<85th percentile for age, with an increase in weight of over 85% of the initial weight loss, which resulted to amenorrhea. In 35 adolescents recovery of menses (two consecutive menstrual cycles) was observed after weight gain (Group A), while 25 girls remained amenorrheic for at least 6 months after complete weight restoration (Group B). The Group A and Group B girls underwent the same examinations at first attendance, and at the end of follow up. Statistical analysis was performed using RStudio, while a t-test was used for comparisons both before and after complete weight restoration. Pearson's correlation coefficient was used in order to examine the correlation between variables and how they affect each other. The level of statistical significance was set at $p<0.05$, while the cut-off point in Pearson's correlation coefficient was set at 0.3.

Results

Table 1 summarizes all the characteristics examined in Group A at first attendance and after menstrual recovery, as well as the same characteristics examined in Group B at first attendance and after complete weight restoration. All data are presented as mean values, with ranges and standard deviation. The mean TSH, FT3 and FT4 levels recorded in Group A were 3.21 ± 0.48 mIU/l, 1.85 ± 0.55 pg/ml and 0.67 ± 0.24 ng/dl respectively at first attendance, and 3.81 ± 0.45 mIU/l, 3.82 ± 0.49 pg/ml and 1.27 ± 0.75 ng/dl respectively at menstrual recovery. In Group B, the mean TSH, FT3 and FT4 levels were 3.08 ± 0.5 mIU/l, 1.61 ± 0.69 pg/ml and 0.59 ± 0.16 ng/dl respectively at first attendance, and 3.4 ± 0.55 mIU/l, 2.94 ± 0.71 pg/ml and 1.08 ± 0.65 ng/dl respectively at the time of complete weight restoration.

Comparing Group A and Group B at first attendance, no statistically significant difference was found in TSH ($p=0.3$), FT3 ($p=0.15$) and FT4 ($p=0.21$) values; instead on comparing the two groups at the time of complete weight restoration a statistically significant difference was found in TSH ($p<0.001$), FT3 ($p<0.001$) and FT4 ($p<0.01$). Evaluating Group A at first attendance and at the time of menstrual recovery, statistically significant differences were found in TSH ($p<0.001$), FT3 ($p<0.001$) and FT4 ($p<0.001$) levels between the two time points; similarly, statistically significant differences in TSH, FT3 and FT4 ($p<0.001$) levels were found in Group B when comparing the values at first attendance and after complete weight restoration. Table 2 summarizes all the comparisons between variables of Group A and Group B at first attendance and after complete weight restoration.

To explore the correlations between the variables contemplated in this study, we used Pearson's correlation coefficient. At the time of menstrual recovery, in Group A, total body fat mass (%) was found to be significantly positively correlated with FT3

Table 1 Group A and Group B characteristics at time of first attendance and after menstrual recovery and complete weight restoration respectively. All data are presented as mean values, with ranges and standard deviation.

	GROUP A AT FIRST ATTENDANCE		GROUP A AT MENSTRUAL RECOVERY		GROUP B AFTER WEIGHT RESTORATION		*GROUP B AT FIRST ATTENDANCE*	
	Mean ± SD	Ranges	Mean ± SD	Ranges	Mean ± SD	Ranges	Mean ± SD	Ranges
Age at menarche (years)	12.61 ± 0.34	12-13.5	-	-	12.56 ± 0.32	12-13.11	-	-
Age at first attendance (years)	16.83 ± 0.75	15-18	-	-	17.03 ± 0.79	15.7-19	-	-
Time from last menstrual period (months)	16.74 ± 2.46	11-22	-	-	21.24 ± 2.74	17-27	-	-
Total weight loss before first attendance (Kg)	8 ± 1.09	6-11	-	-	10.32 ± 1.77	7-14	-	-
Total weight gain (Kg)	-	-	7.51 ± 1.52	5-13	-	-	8.24 ± 1.92	5-12
Time needed for weight gain and BMI normalization (months)	-	-	13.34 ± 2.87	8-24	-	-	13.77 ± 2.98	9-26
Time from diagnosis of AN (months)	12.57 ± 2.38	7-18	-	-	16.52 ± 2.41	12-23	-	-
Physical activity (hours/week)	7.23 ± 1.11	5-9	7.4 ± 0.88	6-9	7.57 ± 1.05	6-10	7.84 ± 1.07	6-10
BMI (Kg/m^2)	16.95 ± 0.64	15.12-18.27	19.58 ± 0.62	18.6-21.4	16.75 ± 0.71	15.32-17.6	19.51 ± 1.13	15.39-21
Waist circumference (cm)	61.11 ± 1.79	57-64	65.77 ± 1.82	63-69	60.16 ± 2.72	55-65	62.92 ± 2.56	55-67
Hip circumference (cm)	78.17 ± 2.53	73-83	81.69 ± 1.92	78-86	77.48 ± 3.24	72-83	81.32 ± 3.66	72-87
WC/HC	0.78 ± 0.02	0.74-0.84	0.8 ± 0.02	0.77-0.84	0.78 ± 0.01	0.75-0.8	0.77 ± 0.02	0.74-0.81
Total body fat mass (%)	17.65 ± 0.82	15.6-19.2	22.57 ± 2.39	20.1-31.4	17.26 ± 0.77	15.4-18.3	18.96 ± 0.8	16.6 – 20.2
Total body fat mass (kg)	8.62 ± 0.71	7.35-10.42	12.73 ±1.48	10.5-19.18	8.53 ± 0.8	7.39-10.06	10.84 ± 0.96	7.47-12.35
Trunk fat mass (%)	17.36 ± 0.84	14.5-18.8	22.22 ± 1.92	20-28.6	16.91 ± 1.12	12.5-18.1	18.27 ± 1.03	14.7-19.4
Trunk fat mass (kg)	3.69 ± 0.48	3.11-4.89	4.87 ± 0.73	3.87-7.34	3.55 ± 0.62	2.7-4.84	3.88 ± 0.47	2.84-5.13
Free fat mass (kg)	40.52 ± 3.15	34.74-46.77	43.91 ± 3.35	35-49.1	40.65 ± 2.62	36.85-45.43	46.28 ± 3.18	37.53-51.9
Free fat mass (%)	82.38 ± 0.79	80.8-84.4	77.51 ± 2.46	68.6-80	82.7 ± 0.83	80.9-84.6	81.08 ± 0.82	79.8-83.4
Trunk/extremities fat *ratio*	0.75 ± 0.02	0.71-0.82	0.85 ± 0.04	0.81-0.97	0.74 ± 0.03	0.64-0.77	0.76 ± 0.03	0.67-0.79
FSH (mIU/ml)	3.97 ± 0.62	2.4-5.3	4.87 ± 0.62	3.7-6.2	3.79 ± 0.72	2.8-5.6	4.37 ± 0.68	2.9-5.9
LH (mIU/ml)	3.06 ± 0.51	2.1-4.2	4.97 ± 0.62	3.8-6.3	3.1 ± 0.57	1.2-3.9	3.48 ± 0.46	2.3-4.3
E2 (pg/ml)	16.74 ± 3.33	9-26	34.43 ± 7.52	24-52	16.28 ± 2.95	11-23	18.96 ± 2.98	12-24
PRL (ng/ml)	13.51 ± 3.06	8-19	13.69 ± 2.51	8-19	12.76 ± 1.96	10-17	12.92 ± 2.25	7-17
TSH (mIU/l)	3.21 ± 0.48	2.3-4.1	3.81 ± 0.45	2.6-4.5	3.08 ± 0.5	1.89-3.8	3.4 ± 0.55	2.21-4.2
FT3 (pg/ml)	1.85 ± 0.55	0.75-4.9	3.82 ± 0.49	2.96-5.76	1.61 ± 0.69	0.44-4.7	2.94 ± 0.73	2.1-5.1
FT4 (ng/dl)	0.67 ± 0.24	0.03-1.45	1.27 ± 0.75	0.76-2.05	0.59 ± 0.16	0.06-1.14	1.08 ± 0.65	0.57-1.75
Insulin (μU/ml)	4.41 ± 0.42	3.6-5.41	5.93 ± 0.53	4.73-6.91	4.44 ± 0.45	3.89-5.6	4.87 ± 0.54	4.06-6.1
Leptin (ng/ml)	2.88 ± 0.44	2-4.1	3.29 ± 0.52	2.4-4.6	2.76 ± 0.39	2.1-3.4	3.01 ± 0.32	2.3-3.7

BMI: body mass index, **WC/HC**: waist circumference/hip circumference, **FSH**: follicle-stimulating hormone, **LH**: luteinizing hormone, **E2**: 17b-estradiol, **PRL**: prolactin, **TSH**: thyroid-stimulating hormone, **FT3**: free triiodothyronine, **FT4**: free thyroxine

(r=0.566, p<0.001) and FT4 (r=0.523, p<0.01), but not with TSH (r=0.272, p>0.05); at the same time point, trunk fat mass (%) was also found to be significantly positively correlated with FT3 (r=0.477, p<0.01) and FT4 (r=0.376, p<0.05), but not with TSH (r=0.235, p>0.05). Furthermore, at the time of menstrual recovery, the trunk/extremities fat *ratio* was statistically significantly positively correlated with FT3 (r=0.586, p<0.001) and FT4 (r=0.512, p<0.01), but not with TSH (r=0.175, p>0.05), showing that, in the Group A girls, distribution of fat in the trunk rather than in the extremities was associated with greater improvements in FT3 and FT4 levels. Finally, at the same time point, leptin levels were found to be statistically significantly positively correlated with FT3 (r=0.609, p<0.001) and FT4 (r=0.57, p<0.01). No statistically significant correlation for the above variables in Group A was found at time of first attendance. On exploration of the correlations between these variables in Group B, after complete weight restoration, we found that total body fat mass (%) was positively correlated, to a statistically significant degree, with FT3 (r=0.309, p<0.05) and FT4 (r=0.395, p<0.05), but not with TSH (r=0.221, p>0.05), while trunk fat mass (%) was significantly positively correlated with FT3 (r=0.501, p<0.01) and FT4 (r=0.333, p<0.05), but not with TSH (r=0.199, p>0.05). In the Group B girls at the time of complete weight restoration, the trunk/extremities fat *ratio* was found to be positively correlated, albeit not statistically significantly, with FT3 (r=0.289, p>0.05), FT4 (r=0.201, p>0.05) and TSH (r=0.191, p>0.05). Finally, at the same time point, leptin levels were statistically significantly positively correlated

Table 2 Comparison of Group A and Group B characteristics at the time of first attendance and after menstrual recovery and complete weight restoration respectively.

	GROUP A AT FIRST ATTENDANCE	GROUP A AND B AFTER COMPLETE WEIGHT RESTORATION	GROUP A AT FIRST ATTENDANCE AND AFTER MENSTRUAL RECOVERY	GROUP B AT FIRST ATTENDANCE AND AFTER COMPLETE WEIGHT RESTORATION
	p-value	p-value	p-value	p-value
Age at menarche (years)	0.67	-	-	-
Age at first attendance (years)	0.39	-	-	-
Time from last menstrual period (months)	<0.001*	-	-	-
Total weight loss before first attendance (Kg)	<0.001*	-	-	-
Total weight gain (Kg)	-	0.06	-	-
Time needed for weight gain and BMI normalization (months)	-	0.07	-	-
Time from diagnosis of AN (months)	<0.001*	-	-	-
Physical activity (hours/week)	0.06	0.10	0.47	0.27
BMI (Kg/m²)	0.27	0.78	0.02*	<0.001*
Waist circumference (cm)	0.13	<0.001*	<0.001*	<0.001*
Hip circumference (cm)	0.38	0.65	<0.001*	<0.001*
WC/HC	0.27	<0.001*	<0.001*	0.53
Total body fat mass (%)	0.06	<0.001*	<0.001*	<0.001*
Total body fat mass (kg)	0.67	<0.001*	<0.001*	<0.001*
Trunk fat mass (%)	0.09	<0.001*	<0.001*	<0.001*
Trunk fat mass (kg)	0.36	<0.001*	<0.001*	<0.001*
Free fat mass (kg)	0.86	<0.001*	<0.001*	<0.001*
Free fat mass (%)	0.14	0.01*	<0.001*	<0.001*
Trunk/extremities fat *ratio*	0.07	<0.001*	<0.001*	<0.01*
FSH (mIU/ml)	0.32	0.01*	<0.001*	<0.001*
LH (mIU/ml)	0.78	<0.001*	<0.001*	<0.001*
E2 (pg/ml)	0.57	<0.001*	<0.001*	0.05
PRL (ng/ml)	0.25	0.22	0.8	0.79
TSH (mIU/l)	0.30	<0.001*	<0.001*	<0.001*
FT3 (pg/ml)	0.15	<0.001*	<0.001*	<0.001*
FT4 (ng/dl)	0.21	0.01*	<0.001*	<0.001*
Insulin (μU/ml)	0.75	<0.001*	<0.001*	<0.001*
Leptin (ng/ml)	0.28	0.01*	<0.001*	0.15

BMI: body mass index, **WC/HC**: waist circumference/hip circumference, **FSH**: follicle-stimulating hormone, **LH**: luteinizing hormone, **E2**: 17b-estradiol, **PRL**: prolactin, **TSH**: thyroid-stimulating hormone, **FT3**: free triiodothyronine, **FT4**: free thyroxine. * Statistically significant difference (p<0.05).

with FT3 (r=0.59, p<0.001) and FT4 (r=0.511, p<0.01). In this group no statistically significant correlation was found for the above-mentioned variables at the time of first attendance. Table 3 summarizes the evaluation of the variables examined with Pearson's correlation coefficient.

Discussion

Anorexia nervosa is known to lead to hypothalamic-pituitary-thyroid axis changes typical of the "euthyroid sick syndrome". In this case, TSH levels are usually normal [5,14], although low TSH levels have been reported in some studies [15], while FT3 and FT4 levels are low. The low T3 levels are a result of peripheral deiodination of T4 to rT3 rather than T3. The presence of carbohydrates appears to be important in stimulating the peripheral conversion of T4 to active T3. Interestingly, a decrease in thyroid hormone levels could contribute to the low REE observed in AN, which serves to preserve energy for vital functions [16]. Administration of exogenous thyrotropin-releasing hormone leads to blunted response of TSH in more than 50% of girls with AN [17]. As reported by many studies, weight gain and complete weight restoration leads to normalization of thyroid hormones [16,18]. This was also shown in our study. At the time of first attendance, levels of FT3 and FT4 were below normal range both in the Group A and in the Group B girls, while at the same time TSH levels were within normal range for both groups. At this time point, no statistically significant difference in FT3, FT4 and TSH levels was found between the Group A and Group B girls. After the end of the refeeding

Table 3 Pearson's correlation coefficient analysis after complete weight restoration in Group A and Group B.

GROUP A AFTER COMPLETE WEIGHT RESTORATION AND MENSTRUAL RECOVERY									
	Total body fat mass (%)	Total body fat mass (Kg)	Trunk fat mass (%)	Trunk fat mass (Kg)	TSH (mIU/l)	FT3 (pg/ml)	FT4 (ng/dl)	Trunk / extremities fat *ratio*	Leptin
Total body fat mass (%)									
Total body fat mass (Kg)	r= 0.797*, p<0.001**								
Trunk fat mass (%)	r= 0.923*, p<0.001**	r= 0.698*, p<0.001**							
Trunk fat mass (Kg)	r= 0.81*, p<0.001**	r= 0.76*, p<0.001**	r= 0.852*, p<0.001**						
TSH (mIU/l)	r= 0.272, p>0.05	r= 0.201, p>0.05	r= 0.235, p>0.05	r= 0.189, p>0.05					
FT3 (pg/ml)	r= 0.566*, p<0.001**	r= 0.499*, p<0.01**	r= 0.477*, p<0.01**	r= 0.531*, p<0.01**	r= 0.102, p>0.05				
FT4 (ng/dl)	r= 0.523*, p<0.01**	r= 0.486*, p<0.01**	r= 0.376*, p<0.05**	r= 0.361*, p<0.05**	r= 0.099, p>0.05	r= 0.123, p>0.05			
Trunk / extremities fat *ratio*	r= 0.588*, p<0.001**	r= 0.384*, p<0.05**	r= 0.56*, p<0.001**	r= 0.463*, p<0.01**	r= 0.175, p>0.05	r=0.586*, p<0.001**	r=0.512*, p<0.01**		
Leptin	r= 0.042, p>0.05	r=0.101, p>0.05	r=0.097, p>0.05	r=0.013, p>0.05	r=0.247, p>0.05	r=0.609*, p<0.001**	r=0.57*, p<0.001**	r=0.197, p>0.05	
GROUP B AFTER COMPLETE WEIGHT RESTORATION									
	Total body fat mass (%)	Total body fat mass (Kg)	Trunk fat mass (%)	Trunk fat mass (Kg)	TSH (mIU/l)	FT3 (pg/ml)	FT4 (ng/dl)	Trunk / extremities fat *ratio*	Leptin
Total body fat mass (%)									
Total body fat mass (Kg)	r= 0.674*, p<0.001**								
Trunk fat mass (%)	r= 0.389*, p=0.05	r= 0.025 p>0.05							
Trunk fat mass (Kg)	r= 0.357*, p<0.05**	r= 0.499* p=0.011**	r= 0.398*, p=0.049**						
TSH (mIU/l)	r= 0.221, p>0.05	r= 0.188, p>0.05	r= 0.191, p>0.05	r= 0.127, p>0.05					
FT3 (pg/ml)	r= 0.309*, p<0.05**	r= 0.356*, p<0.05**	r= 0.501*, p<0.01**	r= 0.442*, p<0.01**	r= 0.156, p>0.05				
FT4 (ng/dl)	r= 0.395*, p<0.05**	r= 0.323*, p<0.05**	r= 0.333*, p<0.05**	r= 0.385*, p<0.05**	r= 0.127, p>0.05	r= 0.131, p>0.05			
Trunk / extremities fat *ratio*	r= 0.218, p>0.05	r= 0.191, p>0.05	r= 0.375*, p<0.05**	r= 0.288 p>0.05	r= 0.191 p>0.05	r= 0.289 p>0.05	r= 0.289 p>0.05		
Leptin	r= 0.045, p>0.05	r= 0.021, p>0.05	r= 0.168, p>0.05	r= 0.135, p>0.05	r= 0.177, p>0.05	r= 0.59*, p<0.001**	r= 0.511*, p<0.01**	r= 0.158, p>0.05	

TSH: thyroid-stimulating hormone, FT3: free triiodothyronine, FT4: free thyroxine; * Pearson's statistically significant correlation coefficient (r > 0.3); ** Statistically significant difference (p<0.05).

program and on complete weight restoration, adolescents from both groups showed normal FT3, FT4 and TSH values, which, in each group considered separately, were statistically significantly higher than at the time of first attendance. Finally, at the same time point, the Group A adolescents showed statistically significantly higher levels of FT3, FT4 and TSH compared with the Group B girls.

Several hormones and peptides have been shown to affect thyroid hormones in adolescents with AN. A keynote hormone in AN, especially in girls who suffer from amenorrhea, is leptin. In these girls, leptin has been found correlate positively with FT3 and FT4, while exogenous leptin administration has been shown to increase levels of thyroid hormones in adolescents with hypothalamic amenorrhea [19-21]. This was also shown in our study. In both groups, leptin levels were statistically significantly lower at first attendance than at the time of complete weight restoration, and statistically significantly higher in adolescents who recovered their menses compared with girls who remained amenorrheic despite achieving weight restoration. Leptin levels were also positively correlated, to a

statistically significant degree, with FT3 and FT4, but not with TSH, at the time of complete weight restoration, not only in Group A, but also in the Group B adolescents.

Even though weight gain and normalization of BMI is known to restore normal thyroid hormone levels, there is no known study that has examined the role, in thyroid hormone abnormalities, of body composition and fat distribution in specific body regions. The first finding of our study was that although the Group A and Group B girls showed no statistically significant difference in BMI, total body fat mass (Kg and %), trunk fat mass (Kg and %), free fat mass (Kg and %), and trunk/extremities fat *ratio* at first attendance, at the time of complete weight restoration, the Group A adolescents showed statistically significantly higher total body fat mass (Kg and %) and trunk fat mass (Kg and %) levels, as well as a significantly higher trunk/extremities fat *ratio*, while the Group B girls had statistically significantly higher free fat mass levels (Kg and %). In both groups, no statistically significant difference change in BMI was found at the end of the study. Unsurprisingly, the Group A adolescents, who recovered their menses, showed higher levels of fat mass, and fat distribution in the trunk, but lower levels of free fat mass, while the weight increase in the Group B girls tended to be attributable to free fat mass, rather than total body fat mass. Second, in both groups, a statistically significantly positive correlation was found between FT3, FT4 and total body fat mass (Kg and %) and trunk fat mass (Kg and %) at the time of complete weight restoration, but no correlation between TSH and total body fat mass (Kg and %) or trunk fat mass (Kg and %) was found in either group at this time point. It is important to note that, at this time point, the adolescents who regained their menses showed a statistically significantly positive correlation between FT3, FT4 and trunk/extremities fat *ratio*, but not between TSH and trunk/extremities fat *ratio*, in contrast with the girls with persistent amenorrhea, who did not present a statistically significant positive correlation between FT3, FT4 or TSH and trunk/extremities fat *ratio* at this time.

Conclusion

Adolescents with AN are known to present "sick euthyroid syndrome", characterized by decreased levels of FT3 and FT4, and usually normal TSH levels (even though decreased TSH levels have been reported in some girls with AN). Weight recovery leads to normalization of thyroid hormones in these adolescents. We used the PubMed database as a primary source for this research, which appears to be the first known study showing a statistically significantly positive correlation between total body fat mass (Kg and %), trunk fat mass (Kg and %) and FT3 and FT4 levels in adolescents with AN who achieved complete weight restoration (with or without recovery of menses). It also seems to be the first known study to find that distribution of fat mass in the trunk (as expressed by trunk/extremities fat *ratio*) was statistically significantly positively correlated with FT3 and FT4 in girls who recovered their menses, in contrast with adolescents who remained amenorrheic. Further studies, with larger samples, are needed in order to strengthen these results.

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Acknowledgements: *The author(s) declare(s) that there are no acknowledgments regarding the publication of this paper.*

Conflict of interest Statement: *The author(s) declare(s) that there is no funding, or research contracts, or conflict of interests regarding the publication of this paper.*