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OBM Neurobiology

Original Research

Increased Diurnal Salivary Cortisol and Morning Serum Triglycerides and Decreased Apo A1 Concentrations in Children and Adolescents with Clinical Depression

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Academic Editor: Bart Ellenbroek

Special Issue: Behavioral Neuropharmacology

OBM Neurobiology
2020, volume 4, issue 2
doi:10.21926/obm.neurobiol.2002060

Received: March 01, 2020
Accepted: May 20, 2020
Published: June 11, 2020

Abstract

Data available on possible associations of lipids, steroid levels, and depression are conflicting. This study investigated their possible associations in a sample of youth with depression. Ninety-eight youth with depression and individually matched healthy volunteers on the basis of age and sex were enrolled for the study. The two groups had nearly similar body mass index. After fasting overnight, they underwent blood sampling for triglycerides

(TG), total cholesterol, low-density lipoprotein, high-density lipoprotein (HDL)-cholesterol, apolipoprotein A1 (apo A1) and B (apo B), lipoprotein A (LpA), morning cortisol, total testosterone (TT), estradiol (E2), and DHEAS measurements. All participants were evaluated with the semi-structured psychiatric K-SADS-PL interview and filled out the self-rating Children's Depression Inventory (CDI). Salivary cortisol was also determined serially five times a day. Children with depression showed significantly higher levels of plasma TG ($p = 0.006$), lower apolipoprotein A1 ($p = 0.043$), higher morning cortisol ($p = 0.011$) and TT ($p = 0.001$), as well as lower E2 levels ($p = 0.032$) compared to those of controls. Positive correlations for the total sample was observed between TG ($R = 0.24$, $p = 0.018$), TT ($R = 0.35$, $p < 0.001$), and morning cortisol ($R = 0.29$, $p = 0.005$) with the CDI scores. The depression group showed elevated cortisol levels 30 min after awakening, 15:00, 18:00, 21:00, and increased cortisol awakening Response and Area Under the Curve (AUCg) ($p = 0.030$, $p = 0.007$, respectively) compared to those of controls. Higher cortisol and TG levels in children with depression imply an increased metabolic risk, starting early in development; therefore, routine metabolic assessment in this group is of great importance.

Keywords
Depression; adolescents; children; lipids; steroid hormones; salivary cortisol

1. Introduction

Adolescent depression is a serious illness with a high incidence in the general population [1]. It is associated with an increased risk of suicide, emotional and behavioral disorders in adulthood, development of metabolic abnormalities, and generally increased all-cause mortality [2]. The incidence of depression increases from childhood to adolescence, and this may be related to hormonal alterations during puberty, and their effects on the developing brain [3].

Lipids are structural and functional elements of the cell, derived from food and synthesized de novo. Lipids are also considered as precursors of steroid hormone synthesis [4].

Altered lipoproteins and apolipoproteins concentrations have been found to be associated with the trait and measures of the state of depression in various adult populations. However, data available to date are contradictory, as several studies have demonstrated a disordered lipid profile in these patients [5-13], while in other studies, the above finding has not been confirmed [14-18].

The literature highlights the effect of steroid hormones, such as estrogen and androgen administration on depressive disorders [19, 20]; however, the association of endogenous gonadal hormones with mood changes has not been elucidated.

A few reports, particularly focusing on the relations between endogenous estrogens and depression, do not, for the most part, describe a statistically significant correlation [21-23]. The relationship between endogenous androgen and depressed mood also remains unclear, as most studies show the absence of any correlation between these factors in younger women [21, 23-27], or in special psychiatric populations [28-30].

However, research investigating the association between the activity of the hypothalamic-pituitary-adrenal axis (HPA) and depression or depressive symptoms has shown more consistent

results; several reports have indicated elevated concentrations of cortisol in serum and saliva of depressed patients [31-33].

Studies focusing on adolescents are scarce, and thus, do not lead to firm conclusions. Some studies used self-reported questionnaires to describe the increase in triglyceride levels in either clinically depressed adolescents [34] or community samples [35, 36]. Additionally, some studies reported elevated cortisol levels [37-40] as well as lowered testosterone levels [41] to play a role in the presence of depressive symptomatology.

The above biological variables could potentially be accessible biological indicators for depressive disorders due to their relatively easy sampling and low cost of analyses [42].

Likewise, adolescence and puberty could be the ideal natural developmental model for the study of the relationship between depression and the above variables, primarily because, 1. In this age range, the presence of exogenous unhealthy habits (e.g., obesity, smoking, alcohol, diabetes) that can act as confounders is limited in relation to the adult sample, giving us the perspective to lead to more robust correlations [43], 2. Environmental and biochemical factors coexist with the effects of stress on the development of behavioral and emotional disorders [44], and 3. It is a period of increased vulnerability to stressors, as it lacks previous useful experiences to which it can resort. [45].

To address the above-mentioned limitations of previous reports, we thought to examine the lipid and selective hormonal levels in a sample of otherwise healthy & naïve from various confounders, depressed young patients, in relation to mentally healthy subjects. We also examined the role of cortisol, as measured by five daily salivary samples from the subjects, while we hypothesized associations between clinical depression and daily salivary cortisol concentrations.

The strong point of our study is the use of a psychiatric interview instead of a self-report questionnaire for the psychiatric evaluation of cases. Thus, we could provide further information about the association between depression, lipids, and steroid hormones.

The analyses presented here test the hypothesis that depression in children and adolescents contributes to lipoprotein and steroid hormone abnormalities, though we cannot express a perspective about the direction of the relationship.

2. Materials and Methods

2.1 Participants

A total of 98 children and adolescents of Greek origin, in the age range of 8 to 17 years, were recruited. The age range in the clinical group was selected based on the normal distribution of the pubertal period, while the earliest normal onset of puberty in girls is eight years, maturity may be complete at 17 [46, 47]. The youth, as well as their parents, received written information on the purpose and procedures of the study and they gave written informed consent. All of them were interviewed and examined by a pediatrician. A full clinical examination was performed, including the measurements of weight, height, and blood pressure. The body mass index (BMI) was calculated as weight/height^2 (Kg/m^2), whereas BMI z-scores were calculated based on the Greek Growth Charts for age and gender [48]. The subject was excluded if factors such as the presence of systemic physical illness, the use of medication as well as the presence of cardiovascular risk factors (familial hyperlipidemia, diabetes mellitus, hypertension, smoking, coronary artery disease, obesity, and systemic alcohol consumption) were detected during the basic pediatric history and

physical examination. Obese children were excluded from the study based on Cole's international criteria [49]. Another exclusion criterion was any circumstance, revealed through the clinical assessment that mood disorders were due to the direct physiological effect of a substance or a physical illness.

The Research Ethics Committee of the University of Athens approved the investigation, and all clinical investigations were conducted according to the principles expressed in the Helsinki Declaration

2.1.1 Case Subjects ("Depression Group")

Forty-nine children and adolescents (mean age: 13.3 ±2.6 years), with the complete diagnosis of depression, were finally recruited. Specifically, subjects with the diagnoses of major depressive disorder, dysthymic disorder, depressive disorder NOS, as well as mixed anxiety-depression disorder, were included. Such a sample of cases was called the "Depression Group".

The cases were individually matched with healthy control subjects at a 1:1 ratio on the basis of age and gender, during the inclusion period of 01/2009 to 12/2017. There was no significant difference among the groups in terms of BMI z scores, education status, and demographic origin.

2.1.2 Controls

Subjects of the control group were recruited from the general population, during the same time period as the case recruitments, after advertising the survey in various selected media on the internet. Similar to the cases, controls were also interviewed and examined by a pediatrician. Those with psychopathological traits and/or physical conditions potentially affecting the biomarkers measured were excluded from the study.

2.1.3 Recruitment of Depression Cases

The Greek version of Kovacs Depression Inventory [50, 51] was applied, as well as basic pediatric history and physical examination were conducted on 136 children and adolescents. Of all these subjects, 61 children met the screening criteria in the study of CDI score ≥ 15 *, depressive symptoms not to be the direct physiological effect of a general physical condition or substance, absence of cardiovascular risk factors, and consent of the family to carry out blood sampling in the Horemeio Research Laboratory.

In nine cases of children invited to the survey, completing the CDI questionnaire did not yield the necessary 15 cut off threshold, as per the relevant research regarding psychometric properties in Greek population [51]; eight cases of children rated < 15, while in one case, CDI was not filled due to absolute illiteracy. However, these subjects entered the research, as the clinical assessment and their reported symptoms suggested an emotional disorder.

The Greek version of Kiddie-Sads-Present and Lifetime Version diagnostic interview (K-SADS-PL) [52, 53] was taken for 55 of them, as in eight cases, either the children themselves or their families did not wish to cooperate, whereas in one occasion the primary caregiver-child psychiatrist posed objections.

After conducting K-SADS-PL, 49 children were included in the full diagnosis of depressive disorders. In particular, five subjects exhibited early-onset dysthymic disorder, eight subjects

exhibited depression NOS, while 27 subjects exhibited major depressive disorder. Also, in one case, adjustment disorder with depressive mood was observed. Further, the criteria for the diagnosis of mixed anxiety depressive disorder were met in seven cases. Consequently, the above 49 subjects were involved in the studied sample of the depression group.

The recruitment process mentioned above is presented in Figure 1**.**

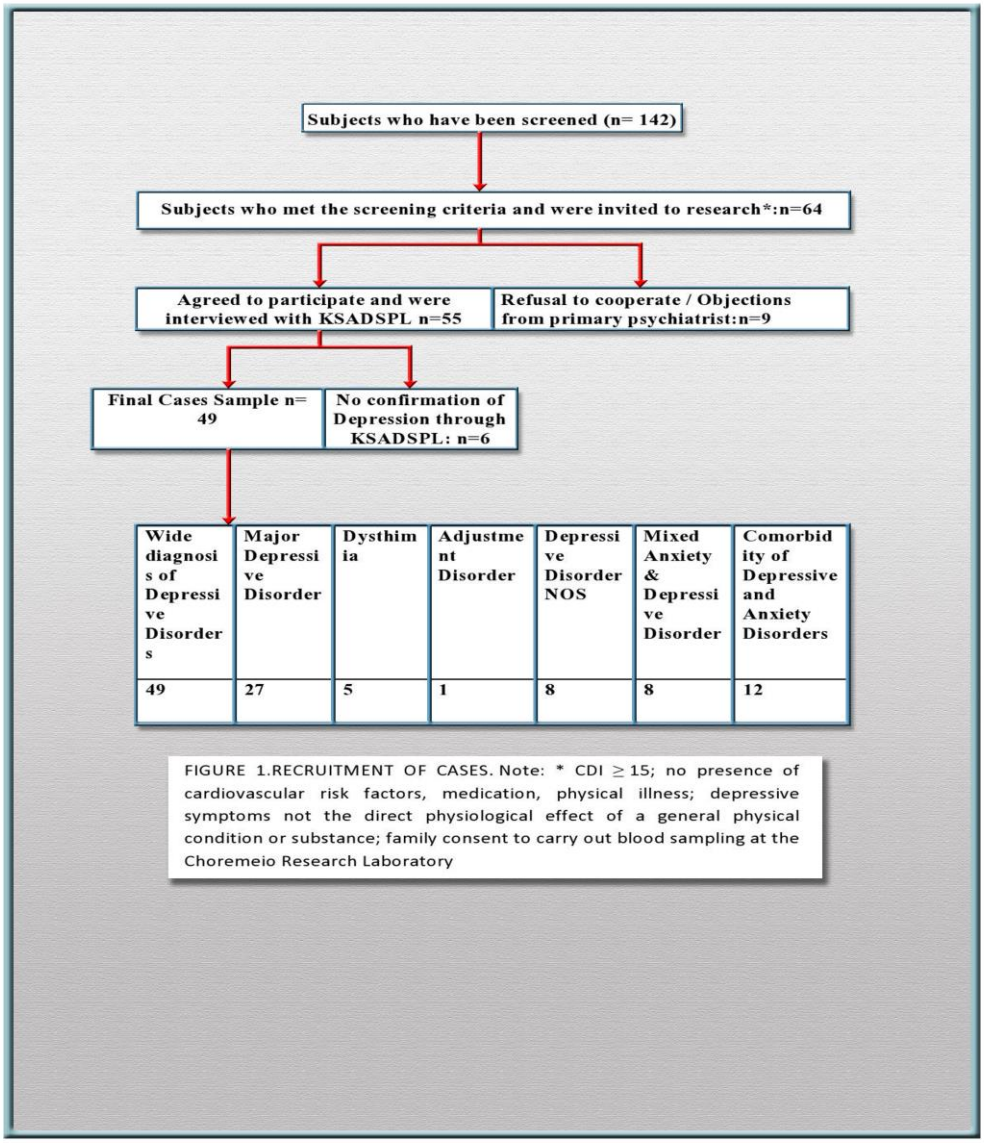

Figure 1 Recruitment of Depression Group.

2.2 Lipids and Steroid Hormones Measurements

Blood samples were drawn strictly between 8-9 am after a 12-hour fasting period.

The following lipidemic factors were determined as follows:

Lipidemic factors: total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, lipoprotein A, apo A1, and apo B.

Hormonal factors: total testosterone, estradiol, DHEAS, and serum cortisol.

Measurement of Lipidemic and Hormonal factors:

Plasma aliquots were stored at–80 °C until analysis. Plasma samples were thawed and equal volumes of plasma from the two groups (depressed and healthy control) were pooled.

The measurements of serum levels of lipids were performed using the Cobas 6000 Chemistry Analyzer (Roche Diagnostics GmbH, Mannheim, Germany).

Serum levels of total testosterone, DHEA-S and estradiol were measured on an Immulite 2000 analyzer (Siemens Healthcare Diagnostics Products Ltd., Llanberis, Gwynedd LL55 4EL, UK) using two-site chemiluminescent immunometric assays with analytical sensitivities for total testosterone (0.20 ng/mL), for DHEA-S (3 μg/dL), and for estradiol (15 pg/mL). The intra-assay and inter-assay precision CVs for total testosterone have a range of 5.1–16.3%, and those for DHEA-S and estradiol were 4.9–13% and 4.3–16%, respectively.

For the salivary cortisol measurements, the following neuroendocrine parameters were assessed in all participants: 1) diurnal variation of salivary cortisol, 2) salivary cortisol area under the curve with respect to ground (AUCg) and with respect to an increased level (AUCi), for "wake to bed" period, as a measure of total cortisol output [54], and 3) salivary cortisol awakening response (CAR) as shown by the increase in cortisol from waking to 30 min later.

To assess the diurnal variation of cortisol, five saliva samples were collected from participants during the day (at the time of waking, 30 min after waking, 12:00, 15:00, 18:00, and 21.00). The family was asked to take the sample on Sunday, while the child's awakening time was scheduled between 8.00 and 9.00. Saliva was harvested using cotton and a saliva collection tube (Salivetta, Salivette, Sarstedt, Nuembrecht, Germany).

When calculating AUCg and AUCi formulas for the "awake to bed" period, the "wake-up" time was set at 08:30 and the "bed" at 21:00.

The detailed instructions were given by the investigator to the young and his family both verbally as well as in the form of written instructions. In addition, the investigator demonstrated face-to-face the process to ensure proper use of the salivette. To increase compliance with the instructions, the sampling process was reminded to parents once again by telephone before the day of sampling. In addition, each salivette had a label that reminded the exact time and sequence of sampling.

The youth had to fast for at least 30 min before sampling the saliva. On the day the samples were taken, he had to be at home and did not have to do physical exercise, intense play, big meals, or be in unusual situations. In addition, he should not have taken alcohol, smoked, used contraceptives, or other medicines.

For sampling, the youth had to put the cotton in his mouth for two minutes or chew it for 1–2 min.

The cotton was repositioned in the plastic tube, in the same position, after closing the cap and stored in a refrigerator specifically at 0–4°C. Parents had to bring the salivette to the researcher within three days. Saliva cortisol was extracted from the cotton by centrifuging the plastic tubes and cotton at 1000 g for 8 min so as to separate the saliva into the outer tube. The cotton smear was then discarded, and all samples were placed in a laboratory refrigerator at –80 °C.

Serum and salivary levels of cortisol were measured on an automated analyzer Cobas e411-Roche Diagnostics (GmbH, Mannheim) by electrochemiluminescence immunoassay with an analytical sensitivity of 0.054 μg/dL and intra-assay and inter-assay precision CVs of 3.0–11.8%.

Estradiol was not measured in the girls who participated in the study, as the menstrual cycle had not been recorded.

2.3 Psychiatric Assessment

The evaluation and diagnosis of depressive disorder and other comorbid psychiatric disorders were conducted by the same child psychiatrist, using the Greek version of the semi-structured psychiatric interview K-SADS-PL (version 1.0, June 2001) [52, 53], after the screening of children and adolescents with depression using the Greek version of Covacs Depression Inventory (CDI) [50, 51]. Diagnosis of clinical depression and exclusion of other disorders was made according to DSM-IV criteria (American Psychiatric Association, 1994), as the study was designed and started in 2009, four years before DSM-V publication.

The inclusion criteria were the detection and diagnosis of the following disorders: a. major depressive disorder (296.xx); b. dysthymic disorder (300.4); c. depressive disorder not otherwise specified (311); d. adjustment disorder with depressive mood (309.0); e. mixed anxiety-depressive disorder (DSM IV TR, Annex B).

2.4 Statistical Analysis

Variables were first tested for normality using the Kolmogorov-Smirnov criterion. Normal variables are presented with a mean (standard deviation), while skewed variables are presented with median and interquartile range (IQR). Qualitative variables are expressed as absolute and relative frequencies. For the comparison of means, an independent Student's t-test was used. Chi-square was used for the comparison of proportions. The association of CDI with biochemical data was explored using Pearson's and Spearman's correlation coefficients. In order to explore differences in biochemical data between the depression and the control groups, conditional logistic regression analyses were performed. Odds ratios with their 95% confidence intervals were computed from the results of the conditional logistic regression analyses. P values reported are two-tailed. Due to the large number of comparisons presented in the text, the Bonferroni correction was not set for all of them because this could lead to a high rate of false negatives [54]. Thus, the statistical significance was set at 0.05.

Following the procedures of Pruessner et al. [55], and Fekedulegn et al. [56], to check the daily cortisol secretion, and the pattern of these changes over the day, the "AUCg" and "AUCi" formulae were used, respectively. These formulae represent two distinct variables of 24-hour cortisol secretion; while AUCg represents the sum of basal cortisol excretion and its change over time (cortisol levels in awakening, plus changes from waking up to 21:00), AUCi can be considered as an indicator of the sensitivity of cortisol response over the day (changes from waking up to 21:00, minus cortisol levels of waking). The analysis was conducted using SPSS, version 22.00.

3. Results

Forty-nine children with depression were matched for sex and age with 49 controls (1:1 matching).

Characteristics of the depression and control groups are listed in Table 1. Mean values for CDI were greater for children and adolescents in the depression group, and these two study groups were similar in terms of BMI z scores. Missing values analysis was not included in the study.

Table 1 Sample characteristics presented by the control and depression groups.

	Control N = 49	**Depression N = 49**	
	N (%)	N (%)	P
Sex of the child			
Boy	17(34.7)	17(34.7)	>0.999¶
Girl	32(65.3)	32(65.3)	
Age (years), mean (SD)	13.3(2.4)	13.3(2.6)	0.955‖
BMI z-score**,** mean (SD)	0.33 (1.11)	0.63(1.56)	0.259‖
CDI	8.7(4.9)	21.0(9.1)	<0.001‖

Note: ‖: Student's t-test; ¶: chi-square test; BMI: body mass index; CDI: Covacs Depression Inventory.

As defined from the results of conditional logistic regression analyses, the depression group was accompanied by higher levels of triglycerides, total testosterone, and serum cortisol and with lower levels of apo A1 and estradiol (Table 2). No difference was observed for the rest of the biochemical data concerning the depression and control groups.

Table 2 Results from conditional logistic regression analyses for biochemical data.

	Group Control	**Group Depression**		
	Mean (SD)	**Mean (SD)**	**OR (95% CI)**	**P**
CHOL	161.8(26.5)	159.8(30.8)	1(0.98–1.01)	0.707
HDL	61.8(12.6)	54.2(20.5)	0.98(0.95–1)	0.093
LDL	88.5(24.4)	91.7(27.4)	1(0.99–1.02)	0.661
TRIGL	62(24.8)	92.5(48.3)	1.03(1.01–1.06)	**0.006**
APO A1	145.5(17)	136.5(19.4)	0.98(0.95–1)	**0.043**
APO B	78(15.1)	76.1(22.2)	1(0.97–1.02)	0.722
LP A, median (IQR)	7.1(4.5–15.2)	8.1(4.9–23.8)	1(0.98–1.02)	0.874
TT, median (IQR)	0.2(0.2–0.4)	20(15–35.4)	1.05(1.02–1.09)	**0.001**
E_2, median (IQR)*	25.6(20.0–32.0)	5.0(5.0–15.3)	0.94(0.89–0.99)	**0.032**
DHEAS, median (IQR)	123(59.4–190)	133.5(84.3–189.5)	1.00(0.99–1.01)	0.301
SERUM CORTISOL, median (IQR)	8(6.3–14.1)	14.8(9.5–21.7)	1.07(1.02–1.12)	**0.011**

Note: Total CHOl, TRIGl, HDL-C, LDL-C, LP A, APO A1, APO B: mg/dL, E_2: pg/mL, TT: ng/mL, DHEAS: µg/dL, Cortisol: µg/dL. Odds Ratio (95% Confidence Interval); *concerns boys; significant correlations in bold (p<0.05).

Significant positive correlations were found in the total sample between triglycerides, total testosterone, and serum cortisol with respective correlation coefficients of 0.24, 0.35, and 0.29 (Table 3).

Table 3 Correlation coefficients of CDI with biochemical data in the total sample and separately in the control and depression groups.

CDI		**Total Sample**	**Control Group**	**Depression Group**
CHOL	R	0.01	0.09	0.02
	P	0.895	0.555	0.888
HDL	R	–0.16	0.18	–0.02
	P	0.113	0.205	0.884
LDL	R	0.02	0.01	–0.13
	P	0.884	0.964	0.371
TRIGL	R	0.24	–0.01	0.09
	P	**0.018**	0.955	0.523
APO A1	R	–0.16	–0.08	0.00
	P	0.112	0.605	0.978
APO B	R	–0.07	0.03	0.01
	P	0.502	0.84	0.945
LP A	R	0.05	0.02	0.01
	P	0.624	0.916	0.954
TT	R	0.35	–0.13	0.16
	P	**<0.001**	0.382	0.27
E	R	–0.16	0.07	0.11
	P	0.111	0.642	0.457
DHEAS	R	0.17	0.32	0.11
	P	0.106	0.026	0.456
SERUM CORTISOL	R	0.29	0.22	–0.21
	P	**0.005**	0.139	0.163

Note: significant correlations are given in bold ($p<0.05$)

3.1 Neuroendocrinological Assessment of Daily Cortisol Fluctuations

Mean cortisol values on analyzing the saliva as well as the values of the CAR, CAR%, AUCi, and AUCg variables in the two study groups are listed in Table 4.

Table 4 Cortisol measures in the depression and control groups.

	Control Group		**Depression Group**		
	Mean	**SD**	**Mean**	**SD**	P*
Cortisol in awakening	9,9	4,8	10,3	5,1	0,631
30 min after awakening	11,8	6,6	14,1	6,7	**0,035**
12:00	5,0	5,3	4,4	3,9	0,465
15:00	3,6	2,9	4,3	2,4	**0,008**
18:00	2,0	1,0	3,5	1,7	**<0,001**
21:00	1,9	1,2	2,4	0,9	**<0,001**
AUCg	3675,78	1679,35	4439,6	1618,64	**0,007**
AUCi	2425,79	1634,43	2520,8	1502,13	0,502
CAR	48,64	116,1	121,06	304,53	**0,030**
CAR%	1,99	7,57	5,06	6,1	**0,026**

Note: *Mann-Whitney test

The cortisol level on awakening was similar in both groups (p = 0.631), but after 30 min, it was significantly higher in the depression group (p = 0.035) compared to the control group. Additionally, at 15:00, 18:00, and 21:00, cortisol levels were significantly elevated in the depression group, compared with the control group (p = 0.008, p <0.001 and p <0.001, respectively). Also, the mean values of the CAR and CAR% were significantly higher in the depressed group (p = 0.030 and p = 0.026, respectively). The mean AUCg was also found to be significantly higher in the depressed group (p = 0.007), while no significant difference was detected in terms of AUCi between the groups (p = 0.026).

Table 5 shows the correlation of cortisol values at different time points with the CDI questionnaire score in the total sample and separately in the two study groups.

Table 5 Correlation coefficients of CDI with cortisol measures in the total sample and separately in the control and depression groups.

		Total Sample	**Depression Group**	**Control Group**
		CDI	CDI	CDI
Cortisol in awakening	R	0,074	0,198	–0,056
	P	0,483	0,197	0,708

30min after awakening	R	0,14	0,106	0,004
	P	0,175	0,484	0,976
12:00	R	0,144	0,196	0,154
	P	0,164	0,191	0,296
15:00	R	0,163	0,008	–0,068
	P	0,119	0,959	0,655
18:00	R	0,475	0,191	0,076
	P	**<0,001**	0,209	0,621
21:00	R	0,383	0,30	–0,137
	P	**<0,001**	**0,047**	0,351
AUC g	R	0,217	0,161	0,041
	P	**0,043**	0,304	0,793
AUC i	R	0,088	0,129	0,099
	P	0,413	0,410	0,524
CAR%	R	0,161	–0,062	0,146
	P	0,125	0,691	0,329
CAR	R	0,165	–0,084	0,200
	P	0,115	0,586	0,177

In the depression group, only the cortisol value at 21:00 was found to be positively correlated with the score in the CDI questionnaire. In particular, higher cortisol values were found to be associated with higher CDI score values. On the whole, the cortisol values at 18:00 and at 21:00, as well as the AUCg were found to correlate positively with the CDI score.

4. Discussion

In the present study, 49 patients with depression and 49 healthy controls were assessed and compared for TG, total cholesterol, LDL- and HDL-cholesterol, apo A1, apo B, Lp A, morning cortisol, total testosterone, estradiol, and DHEAS.

As defined from the results of conditional logistic regression analyses, the 49 adolescents with depression in comparison with the control group showed significantly higher median plasma TG levels (92.5 ±48.3 vs. 62 ±24.8 mg/dL), lower apo A1 levels (136.5 ±19.4 vs. 145.5 ±17 mg/dL), higher morning cortisol (14.8 (9.5–21.7) vs. 8 (6.3–14.1) mg/dL), higher TT levels (20 (15–35.4) vs. 0.2 (0.2–0.4) mg/dL), and lower E2 levels (5.0 (5.0–15.3) vs. 25.6 (20.0–32.0) mg/dL), respectively. HDL-cholesterol levels tended to be significantly lower in the depression group (54.2 ±20.5 vs. 61.8 ±12.6 mg/dL, P = 0.093).

The CDI questionnaire indicates the severity of the depressive symptoms; therefore, significant positive correlations were observed, for the total sample, between TG, apoA1, cortisol, and TT concentrations and the results of the questionnaire (p = 0.027, 0.048, and 0.018), respectively.

Our finding of increased TG levels in depressed youths is in accordance with the results of Glueck et al. [34], where a large sample of hospitalized clinically depressed adolescents was recruited. Additionally, in the Early Bird study by Jeffery et al. [35], in which 208 healthy children and adolescents participated, the relationship of mood with triglycerides and other variables was also investigated. They observed that the subjects with low mood had higher triglycerides ($r = -0.11$, $p = 0.06$). In the most recent cross-sectional study by Gross et al. [36] involving 208 adolescents, depressive symptoms were measured by the self-completed Epidemiological Studies Depression Scale questionnaire and, similar to our study, positive correlations were observed with triglycerides. Nevertheless, compared to our study, the recruitment of community samples, as well as the assessment of mood through a self-reported questionnaire, were two essential discrepancies in the Early Bird study and the study of Gross et al.

Several studies with adults, using cross-sectional designs, also indicate elevated triglyceride levels in depressed patients [57-59]. However, some studies do not confirm our findings [14, 16]. Study design variations and heterogeneity of study participants are possible explanations for these inconsistent findings. A clear advantage of our study was the use of the semi-structured psychiatric interview K-SADS-PL, instead of the use of a self-report questionnaire for the psychiatric evaluation of cases. Moreover, while our sample of youth is naïve for various confounders that have been shown to affect triglyceride levels, such as comorbidity with other physical conditions [60], obesity [61], smoking [62], and alcohol [63], and this can also be described as an advantage of our study with regard to the other studies.

Cholesterol levels were not found to be different in the case sample compared to controls in our research. This finding is in accordance with that of Apter et al. [64], where the authors, in a large sample of 152 hospitalized adolescents, also did not detect an important association between cholesterol levels and depression severity.

On the contrary, various studies, mostly on adults, describe reduced cholesterol levels in subjects with depression [62, 65, 66]. This discrepancy in our study, is probably due to the comorbidity of some cases, with pathological anxiety· (e.g., eight cases with mixed anxiety depressive disorder, and 12 depression cases comorbidity with anxiety disorders). According to some studies, pathological anxiety appears to be associated with elevated cholesterol levels [67-69], probably, due to noradrenergic hyperactivity [70]. Therefore, this lack of abnormality in cholesterol levels in our samples could be explained and was expected. Apart from that, unlike adults, it is postulated that appetite loss, aggressive and antisocial behavior, and the limited use of meditation in depressed adolescents, could influence cholesterol levels [64]. Furthermore, early depression in adolescence may represent a more severe form of depression than the adult-life disorder, with a heightened biological background [71].

Interestingly, apolipoprotein A1 was significantly reduced in depressed subjects in our study. Based on the evidence so far, apoA1 appears to have a protective function concerning cardiovascular events [72, 73], while low levels have been observed in depressed adults [12, 13, 72]. The reduced apoA1 may also be associated with increased BMI in children [73], but as already mentioned, BMI did not differ between the two groups in our study. Our finding, detected in a sample of depressed children and adolescents, appears to strengthen the hypothesis that reduced apo A1, among other cardiovascular risk factors, possibly mediates the association of depression with the cardiovascular burden of these patients.

Other interesting findings are the heightened serum cortisol levels in the depressed group, as

well as the positive correlation between morning serum cortisol levels and the severity of depressive symptoms. This finding is in agreement with the findings of Morris et al. [74], wherein, the authors argue that the response of the HPA axis to adolescents appear to be correlated with the severity of depressive symptoms. Likewise, in a study by Van den Bergh et al. [39], in which 59 adolescents participated, the increase in the severity of depression symptoms, as per the CDI questionnaire, affected the increase in the daily fluctuation of cortisol. While the majority of studies support the increase in cortisol levels and the over-activity of the HPA axis [31, 32, 37, 40], in few studies, this finding is not confirmed [75, 76]. According to Morris et al., the heterogeneity of the results may be due to the variation in response of the HPA axis determined by the relative superiority of emotional to autonomic elements in the overall depressive phenomenology. In addition to that, the time from waking up until the recording of morning serum cortisol [77], the medication [78, 79], as well as different sources of psychiatric data, i.e., from parents, research staff, teachers, etc. [38], could affect the results. Based on the above limitations, the primary advantages of our study was the use of a K-SADS-PL which is considered as a gold standard to confirm the diagnosis and the severity of major depression according to DSM-IV-TR criteria [50], as well as the strict timing of blood sampling to record morning serum cortisol.

The daily fluctuation of saliva cortisol in the group of depressed subjects was analyzed. The AUCg and AUCi variables were measured as indicators of the overall 24-hour activity of the HPA axis. In particular, AUCg is associated with the estimation of total hormone secretion of cortisol, while AUCi correlates with pattern or rate of change in cortisol levels over time [56]. In our study, the AUCi did not differ significantly between the two groups ($p = 0.502$). This finding suggests a daily rate of change in cortisol levels, which in normal subjects peaked about 30 min after waking up and decreased gradually thereafter.

We detected significantly increased AUCg cortisol in children and adolescents with clinical depression ($p = 0.007$); the AUCg considers both fluctuations in cortisol levels throughout the day (i.e., the change in cortisol levels from one-time point to the next), and the total magnitude of cortisol secretion, i.e., the distance of these measures from the basal level [55, 80]. Thus, our study shows increased cortisol secretion in children and adolescents with depression during a 24-hour period. In addition, the severity of depressive symptoms based on CDI scoring was found to correlate positively with the AUC variable for the total sample. These findings are in agreement with studies on adults that focused on clinically depressed samples, describing increased daily cortisol production through this variable [81, 82].

In this study, children with clinical depression, compared with the controls, showed significantly higher cortisol levels at four-time points, 30 min after waking, at 15:00, at 18:00, and at 21:00 on the day of sampling. In addition, the severity of depressive symptoms as described by the CDI scores correlated positively with cortisol values marked at midday (15:00), and evening (21:00). In accordance with our findings, the data from some studies show that compared to the control group, an increase in cortisol levels is frequently detected in depressive subjects, at various measurement times over 24 h, especially in the evening hours [37, 39, 83, 84]. However, we did not observe any variation in cortisol until 21:00 in the evening, and not later.

We detected a significantly elevated mean CAR, in addition to an increase in the relative increase in cortisol over the first concentration of awakening (CAR%) compared to the control group. It is important to note that the CAR is different from total daily cortisol secretion, calculated as AUCg [85]. It indicates a steep rise in cortisol levels, from the moment of waking up until it is

recorded 30 min later. In healthy individuals of all ages, there is a 38–75% rise in cortisol level at the time of waking up, reaching its maximum level within 30 min [86]. This increase is thought to be associated with awakening, possibly through activation of self-reported memory representations that stimulate the activity of the HPA axis in line with the expected requirements of the upcoming day [87]. Various studies have so far described increased CAR in adult patients with clinical depression [31, 81, 88, 89]. With regard to studies in children and adolescents, the majority of findings show, parallel with our study, an increase in CAR; however, some studies do not confirm this finding. Specifically, Shibuya et al. [90] identified an increased response to cortisol in those subjects with increased depressive symptoms; a community sample of only 18 girls aged 13–16 years was analyzed, which, unlike our study, did not exhibit clinical depression, while mood was recorded through a valid self-completed questionnaire. Also, no control group was used in this study. In addition, in the TRAILS study of Bosch et al., which used a large community sample of over 2,000 preadolescents, the CAR variable only increased in those boys with increased physical symptoms of depression and reduced in those boys with enhanced emotional-cognitive symptoms, but no strong correlations were observed. The overall measurement of depressive symptoms, however, was not associated with changes in the CAR variable [91]. On the other hand, a weak association between CAR and depression, regardless of gender, was found in the study of Dietrich et al., where a large community sample (n = 1,604) was analyzed [92]. In both studies, depression was diagnosed through a self-completed questionnaire rather than a clinical interview.

In summary, in accordance with our findings, an increasing number of studies demonstrate that depressed individuals exhibit a steeper increase in cortisol after awakening (CAR), and higher overall levels during the day, as assessed by the AUCg or individual cortisol estimates at different time points of the day. Diversity in the results could be attributed mainly to: a. comorbidity with pathological anxiety, which, as already mentioned, was observed in 12 individuals of our sample [93], b. a different methodology was followed, with different control for confounders [81], c. the hypothesis that, in samples of the general population, when compared to a sample indicating clinical depression, the activity of the HPA axis may differ [31, 88], and d. different sex ratios in the studied samples [80].

The detection of elevated total testosterone levels in our sample raises questions. Two studies conducted so far in the adolescent population demonstrate, in contrast to our study, an inverse relation of testosterone and negative effects [41, 94]. Additionally, in another study where 40 disruptive youths participated, the correlation between these variables was not observed [38]. Nevertheless, these studies exhibited significant differences compared to our research. In the study by Susman et al. [94], an inverse association was detected only for boys, while the negative effect was subject to evaluation in contrast to clinical depressive disorders in our study. Likewise, in the study by Granger et al. [39], this association was only detected for boys, while the internalizing symptoms were derived from self- and parent-reported questionnaires. In addition, saliva samples were analyzed. Finally, in the study by Scerbo & Kolko [38], the sample consisted of disruptive adolescents, with the majority of participants being boys, wherein, similar to the previous studies, internalizing behavior was evaluated by self-reported questionnaires, and measurements were based on saliva samples. On the other hand, in our research, testosterone levels were evaluated in strictly clinically depressed subjects, with a large proportion of participants being girls; moreover, psychiatric evaluation was conducted through a reliable instrument. Based on the above heterogeneity in the results, it could be postulated that the association of total testosterone and

depressive symptoms in young people without clinical depressive disorder may be due to different biological pathways compared to those people with a clinically significant effect, and the hormone response might be different in girls compared to that in boys.

Our research indicates the absence of an association between estradiol and depression in depressed subjects. This finding is concordant with the previously mentioned favorable effect of exogenous estrogens on depressive syndromes described in some adult studies [19, 20]. However, no consistent correlation has been found in the few studies on endogenous estrogens conducted so far, focusing on various ages in study populations [21-23]. Apart from that, in the present study, estradiol and depression were investigated only in 17 boys, a limited sample to help us draw firm conclusions.

In the present study, plasma TG levels, as well as the morning serum and salivary cortisol, were found to increase in subjects suffering from depression. Based on data so far, we speculate that the increased cortisol levels might increase serum levels of circulating free fatty acids, which in turn stimulate the synthesis of very-low-density lipoprotein (VLDL) in the liver resulting in elevated TG concentrations [95, 96]. Additionally, adverse lipid patterns in depressive disorder are associated with lifestyle-related factors. Adolescents with depression were reported to often eat foods high in energy density compared to healthy controls [97]. This cofactor might be one possible reason for a positive association between plasma TG levels and depression in this study. Nevertheless, understanding of this association in childhood and adolescence still remains unclear and more research is needed [64, 98].

Furthermore, our sample is a non-homogeneous group, and consists of various subtypes of depressive disorder, with possible diversity in phenomenology, course of illness, and etiopathogenesis [99].Therefore, it would be risky to argue for the hypothesis that the biological processes through which various depression subtypes of individuals associate to increased triglycerides levels are similar.

In our study, we also detected a slightly lower level of HDL cholesterol in depressed subjects. Recent evidence suggests that elevated TG levels associated with lowered HDL-cholesterol are a predisposition to atherosclerosis [100, 101]. Based on the findings of this study, TG and HDL-cholesterol measurements may have the potential to serve as screening biomarkers in individuals with depression, to prevent long-term development of cardiovascular events and its complications.

5. Conclusion and Future Directions

This study suggests an association between adverse lipid and steroid hormones patterns and depression in a sample of children and adolescents, matched to age and sex, with healthy controls of equal body mass index. Elevated triglyceride levels, low apo A1 levels, and elevated cortisol levels at various time points of the daily fluctuation further strengthen existing reports that recommend depression as a possible cardiovascular risk factor. Due to the cross-sectional design of this research, we cannot imply a causal relationship between the parameters examined.

An interesting research point is the preliminary findings of Elovainio et al. [102], according to which the relatively rapid increase in triglycerides at an early age may be associated with depressive symptoms in adulthood. We, therefore, postulate that the examination of a long-term association between triglycerides and depression in future studies could provide us with more information about their relationship.

Based on the evidence so far, it remains unclear if cortisol levels represent a neurobiological marker, a vulnerable trait to depressive disorder, or even a "scar" of the depressive episode [80, 89, 103, 104]. Further investigation, including prospective studies with extended study periods, and larger size sample groups are required, to understand the directionality of the above-mentioned associations.

Furthermore, it would be challenging to investigate lipid levels as potential markers of clinical response to antidepressant therapy in adolescents [105, 106].

It is also interesting to note that studies, including ours, which focuses on children and adolescents, show many similarities, but also differences in the biological profiles of depressed youth compared to adults with depression. The weaker correlations detected between low cholesterol, cortisol response in awakening, and depression, likely indicate a different pattern of depression at this age, which also reflects differences in clinical profile, etiology, and prognosis. More research, with individualized methodology, and not "borrowed" from research on adults, could provide answers in this area.

Also, future studies involving larger sample sizes, and prospective design, need to be carried out to determine if the presence of certain symptoms in patients with clinical depression signals a specific biochemical profile accompanied by specific prognosis, etiology, pathogenesis, cardiovascular, as well as therapeutic response.

6. Additional Points

Limitations. In interpreting the present results, we observed a few limitations. First, the pubertal status by the tanner stage of the individuals was not considered; while this important information was assessed in the clinical group, it was not assessed in the control group. Furthermore, no attention was paid to estimating the socioeconomic status of the participants. These variables are widely known to influence the examined link [107, 108]. However, as mentioned before, in our work, both cases and controls were of Greek origin and did not deviate in reference to the BMI, age, and gender, the factors that are known to essentially mediate the association between depression and lipid abnormalities. In addition, the majority of participants, in our study, came from the Northern suburbs of Attica, living nearby the Hospital.

Our sample size was relatively small, although the age and gender matching with controls in a 1:1 ratio was an advantage of our study.

Further, our assumptions concerning the connections between depression and the biochemical pathways were based only on available lipidemic factors, and steroid hormones studied. Parameters such as free testosterone, ACTH, thyroid hormones, cytokines, and other neuroendocrine abnormalities, which are also implicated as factors in depression, were not investigated.

There is evidence that secretion depends on circadian and ultradian periodicity; therefore, cortisol levels should be measured more often [104, 109]. Another limitation of our study was that daily fluctuations of saliva cortisol were measured in only one day, and the exact waking up, and bedtime were not recorded, which are important for assessing CAR and AUC values. Furthermore, basal cortisol concentrations, relative to stress-induced cortisol concentrations, are a weaker measure of HPA activity in children [110].

Finally, while those with depression were clinical cases, the control group consisted of children

of the general population. This difference may prevent us from generalizing the conclusions. However, as noted before, our sample consisted of youth with no remarkable confounding factors such as medication, physical comorbidity, known cardiovascular risk factors, etc.

Acknowledgements

The authors wish to thank Chara Tzavara for her invaluable advice on statistical matters.

Author Contributions

Syros Ioannis conceived the original idea and wrote the manuscript in consultation with Gerasimos Kolaitis and Panagiota Pervanidou. Gerasimos Kolaitis and Panagiota Pervanidou supervised the project. George Chrousos and Charis Liapi provided critical feedback and helped shape the research, analysis and manuscript. Filia Apostolakou assisted with biochemical measurements.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing Interests

The authors state that there are no conflicts of interest regarding the publication of this paper.

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