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Research article

Relationship between lipid metabolism state, lipid peroxidation and antioxidant defense system in girls with constitutional obesity

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Abstract: The study of the molecular mechanisms involved in adolescent obesity formation is important due to the severe and prolonged complications in adulthood. Here, we analyzed the lipid metabolism and peroxidation systems and the relationships between them in girls with constitutional obesity. Thirty-nine adolescent girls 14–16 age with constitutional obesity and twenty-six girls with a normal body mass index (control group) of the same age were examined. Spectrophotometric and fluorometric research methods were used. Constitutional obesity in adolescent girls is accompanied by the development of dyslipidemia (increased concentrations of total cholesterol, triacylglycerols, and very-low-density lipoproteins), as well as by reduced antioxidant defense components (total antioxidant activity, retinol, and the oxidized form of glutathione). In addition, adolescent girls with constitutional obesity had an increased number of correlations in the lipid peroxidation–antioxidant defense system and intersystem correlations, which indicates the insufficient activity of the antioxidant defense system. These results increase our understanding of the pathogenic mechanisms involved in adolescent.

Keywords: obesity; adolescence; lipids; antioxidants; lipid peroxidation

Abbreviations: AOD: antioxidant defense; BMI; body mass index; CD: conjugated; DBP: diastolic blood pressure; GSH: reduced glutathione; GSSG: oxidized glutathione; HDL: high-density lipoproteins; LDL: low-density lipoproteins; LPO: lipid peroxidation; OS: oxidative stress; PUFA: polyunsaturated fatty acids; SBP: systolic blood pressure; SD: standard deviation; SDS BMI: standard deviation score of body mass index; SOD; superoxide dismutase; TAA: total antioxidant

activity; TBAR: thiobarbituric acid; VLDL: low-density lipoproteins; WHO: World Health Organization

1. Introduction

Obesity is a problem in many countries worldwide [1,2]. Special attention needs to be paid to childhood and adolescent obesity because it often progresses and leads to the development of complications in adulthood. In developed countries, up to 25% of adolescents are overweight and 15% are obese [3,4]. The WHO attributes the widespread prevalence of childhood obesity to modern economics and social conditions, which result in an unhealthy diet and a low level of physical activity [5].

Constitutional obesity is the most common form and is characterized by a pronounced imbalance between calories consumed and their expenditure [4,6]. Among adolescents, the constitutional form of obesity is most common in girls [7]. The main predictors of adolescent obesity, in addition to hereditary factors, are hypodynamia, an imbalance in energy metabolism, a burdened family history, and psycho-emotional stress [1,3,6].

Obesity is associated with chronic inflammation of adipose tissue, activation of proinflammatory reactions, dyslipidemia, and oxidative stress development [8–12]. The consequences of oxidative stress progression include the accumulation of cytotoxic compounds, including endogenous aldehydes, which act as mediators of damage and provoke changes in metabolism [2,5,8]. An important role in the defense against damage induced by oxidative stress is played by the antioxidant defense system (AOD), whose components inactivate free radicals, stabilize biological membranes, and prevent the chain of free-radical processes that oxidize organic compounds, primarily unsaturated tissue lipids [13,14]. This latter function is primarily provided by antioxidant enzymes, including superoxide dismutase (SOD), catalase, enzymes of the redox glutathione system, and water- and fat-soluble vitamins [15,16]. The ratio of the activity of oxidative processes and the AOD determines the intensity of metabolism and its adaptive capabilities. Failure of the AOD is characterized by the development of lipid peroxidation syndrome, which leads to cell membrane damage, enzyme inactivation or transformation, the suppression of cell division, and the accumulation of inert polymerization products [17].

Despite numerous studies on changes in the lipid metabolism parameters and the lipid peroxidation system in obesity, there is little information about the biochemical characteristics and relationships between the abovementioned individual components in adolescents with constitutional obesity. Therefore, we analyzed the lipid metabolism and peroxidation systems and the relationships between their parameters in girls with constitutional obesity.

2. Materials and methods

2.1. Design of study

Thirty-nine adolescent girls (mean age: 15.47 ± 1.08 years) living at Irkutsk region with constitutional obesity were examined. As a control group, we used data from 26 practically healthy adolescent girls of the same age (mean age: 15.45 ± 1.09 years). Height (cm), body weight (kg), body mass index (BMI; kg/m^2), standard deviation score of body mass index (BMI SDS), waist

circumference (cm), systolic (SBP) and diastolic (DBP) blood pressure (mmHg), and fasting glycemia level (mmol/L) were measured. The standard deviation of the BMI for diagnosing constitutional obesity was determined using the Antro Plus World Health Organization program (2009). The diagnosis of obesity was established when the SDS BMI was more than +2.0 [18]. Criteria for inclusion in the group with constitutional obesity were as follows: SDS BMI 2.0–2.5; exclusion of acute or exacerbation of chronic diseases at the start of examination or 1 month prior; permanent residence of the girls in the territory of Irkutsk region; signing of informed consent to be included in the study by parents or legal representatives, as well as by teenagers over 15 years of age. Criteria for exclusion from the group of patients included physical development delay; body weight deficiency; genetic and symptomatic forms of obesity; taking medications that potentially affect body weight and estimated biochemical characteristics.

The basic characteristics of the groups are shown in Table 1.

Table 1. Clinical characteristics of girls with constitutional obesity (Mean ± SD, Median, quartile (25–75%)).

Parameters	Control (n = 26)	Patients (n = 39)
Age, years	15.45 ± 1.09	15.47 ± 1.08
	15 (15–16)	15 (15–16)
Height, cm	159 ± 0.06	162 ± 0,11
	160 (155–165)	162 (159–168)
Body weight, kg	51.39 ± 3.86	78.47 ± 14.04 *
	50 (48.8–53)	77.2 (72–84)
BMI, kg/m^2	20.16 ± 1.4	30.31 ± 3.57 *
	19.56 (19.5–21.12)	30.3 (30.1–34.4)
SDS BMI	0.18 ± 0.1	2.91 ± 0.41 *
	0.44 (−0.61–0.72)	3.03 (2.55–3.19)
Waist circumference, cm	60.79 ± 3.07	87.7 ± 12.29 *
	61 (63.5–65.5)	91 (74.1–98)
SBP, mmHg	106.58 ± 8.54	111.26 ± 9.91
	110 (98.12–111.23)	104.87 (102.6–115.8)
DBP, mmHg	63.24 ± 4.2	69.58 ± 6.92
	64.38 (60.6–65.74)	65.21 (64.39–72.56)
Glucose, mmol/L	3.89 ± 0.62	4.11 ± 0.66
	3.61 (3.09–4.56)	3.74 (3.3–5.2)

* Statistically significant difference.

The analysis of anthropometric data in adolescent girls with constitutional obesity showed a statistically significant increase in the main indicators: weight ($p < 0.0001$), BMI ($p < 0.0001$), SDS BMI ($p < 0.0001$), and waist circumference ($p < 0.0001$) in adolescent girls in comparison with the control group (Table 1). There were no statistically significant differences in the other clinical characteristics ($p > 0.05$).

2.2. Ethics approval

The study was conducted in accordance with the Helsinki Declaration of the World Medical Association (1964, 2013 ed.) and approved by the Biomedical Ethics Committee at the Scientific Centre for Family Health and Human Reproduction, Russia (No. 9 dated 10.08.2014).

2.3. Biochemical measurements

The study material was blood serum, plasma, and hemolysate. Blood was taken for analysis from the ulnar vein, taking into account the generally accepted requirements.

Blood plasma and serum were obtained by blood centrifugation at 3000 g for 5 min at 4 ℃. Samples were stored at −80 ℃ until measurements. Dynamic monitoring of patients was carried out throughout the entire period of their stay in hospital.

The total cholesterol (TC), high-density lipoprotein (HDL), and triglycerides (TG) levels were determined in serum by photometry using Cormay kits on the BTS-330 automatic analyzer «BioSystems» (Spain). The content of low-density lipoproteins (LDL) was calculated using the formula: LDL = TC – (HDL + VLDL), where the VLDL (very low-density lipoproteins) level = TG/2.2. Lipid spectrum indicators were assessed following the recommendations of the Russian Scientific Society of Cardiology and the Association of Pediatric Cardiologists of Russia (2012) [19]. According to these criteria, elevated levels were considered to be as follows: TC > 4.39 mmol/L, LDL > 2.84 mmol/L, and TG > 0.99 mmol/L. High levels were: TC > 5.19 mmol/L, LDL > 3.34 mmol/L, and TG > 1.3 mmol/L, HDL < 1.30 mmol/L was considered to be low [19].

To assess the states of the lipid peroxidation (LPO) and AOD systems, the contents of the following components were determined: conjugated dienes (CDs) [20], thiobarbituric acid reactants (TBARs) [21], total antioxidant activity (TAA) of the blood [22], α-tocopherol and retinol [23], reduced (GSH) and oxidized (GSSG) glutathione [24], as well as superoxide dismutase (SOD) activity [25].

The method for determining the content of CDs in blood plasma is based on their intense absorption in the region of 232 nm [20]. The coefficient of molar absorption ($K = 2.2 \times 10^5$ M^{-1} C^{-1}) was used to convert absorption units to μmol/L. TBAR levels were determined in plasma by reaction with thiobarbituric acid followed by measurement of fluorescence intensity at 515 nm (excitation) and 554 nm (emission) [21]. TBARs concentration is expressed in μmol/L. To assess the TAA of blood plasma, a model system, which is a suspension of chicken egg yolk lipoproteins, was used to assess the ability of plasma to inhibit the accumulation of TBARs in suspension. LPO was induced by the addition of $FeSO_4{*}7H_2O$ [22]. TAA is expressed in equivalent units. α-Tocopherol and retinol levels were detected in the blood plasma [23]. The samples were incubated for 20 min and measurement was performed at λ = 294 nm (excitation) and λ = 330 nm (emission) for α-tocopherol and at λ = 335 nm (excitation) and λ = 460 nm (emission) for retinol. Concentrations are expressed in μmol/L The content of GSH and GSSG [24] and the activity of SOD [25] were determined fluorometrically in erythrocytes (hemolysate). GSH and GSSG levels were detected at identical conditions at λ = 350 nm (excitation) and λ = 420 nm (emission) [24]. Concentrations are expressed in mmol/L. The method for determining the activity of SOD is based on the inhibition of the autooxidation of adrenaline into adrenochrome at pH 10.2. SOD activity was measured at λ = 320 nm. The amount of SOD required to inhibit the rate of adrenaline autooxidation by 50% was taken as

the conventional unit of enzyme activity [25]. SOD values were measured using a Shimadzu RF-1501 spectrofluorophotometer (Japan) and activity is expressed in equivalent units. This work was carried out using the equipment of the Centre of Collective Usage "Center for the Development of Progressive Personalized Health Technologies", Scientific Centre for Family Health and Human Reproduction Problems, Irkutsk.

2.4. Statistical analysis

An integrated system for complex statistical analysis and data processing in the STATISTICA 6.1 program was used (Stat Soft Inc., USA). To determine the proximity to the normal distribution of quantitative features, the visual-graphical method and the Kolmogorov–Smirnov test with amendments by Lilliefors and Shapiro–Wilk were used. Due to the abnormal distribution of data, the differences in quantitative indicators between the study groups were assessed using the Mann–Whitney U-test. Spearman correlation analysis was used to analyze intra-group relationships between quantitative traits. The significance level for testing statistical hypotheses was $p < 0.05$.

3. Results

In the analysis of lipid metabolism, adolescent girls with constitutional obesity showed increased values of TC (0.14-fold here $p = 0.01$), TG (1.21-fold here times; $p < 0.0001$), and VLDL (1.21-fold here $p < 0.0001$) compared to the control group (Figure 1).

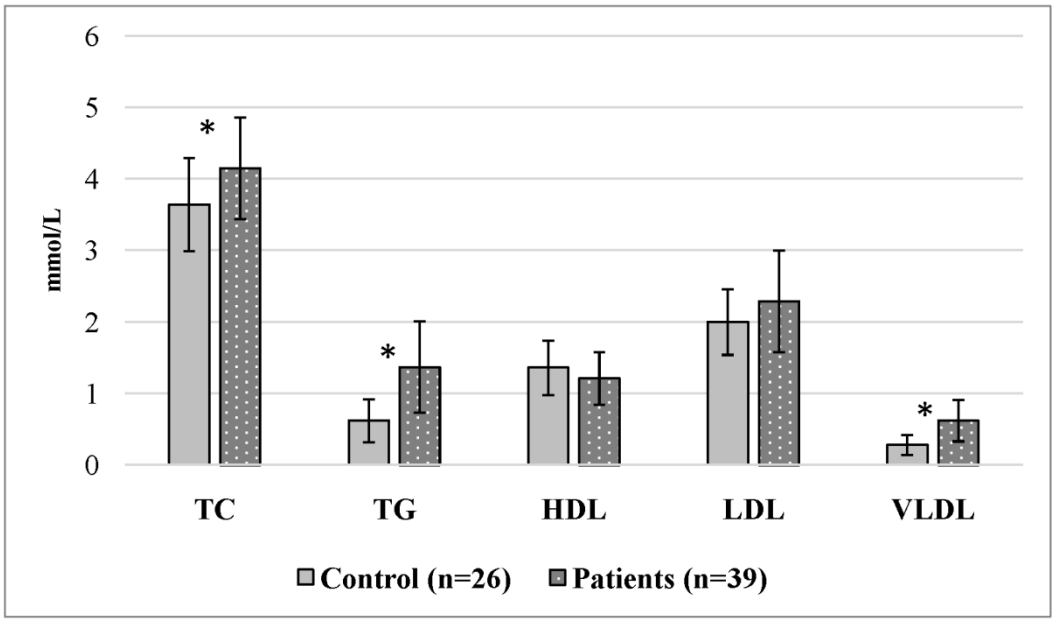

Figure 1. The level of serum lipids in girls with constitutional obesity. * statistically significant difference.

The study of the lipid peroxidation process parameters found that the level of the primary LPO products, CDs, significantly increased in girls with obesity compared with the control group by

0.5-fold here (p = 0.0018) but there was no difference in the TBARs content (Figure 2). In obese girls, the antioxidant defense system was decreased compared to the control group: TAA 0.34-fold here (p = 0.0252), retinol 2.87-fold here (p < 0.0001), and GSSG 0.22-fold here (p = 0.0043).

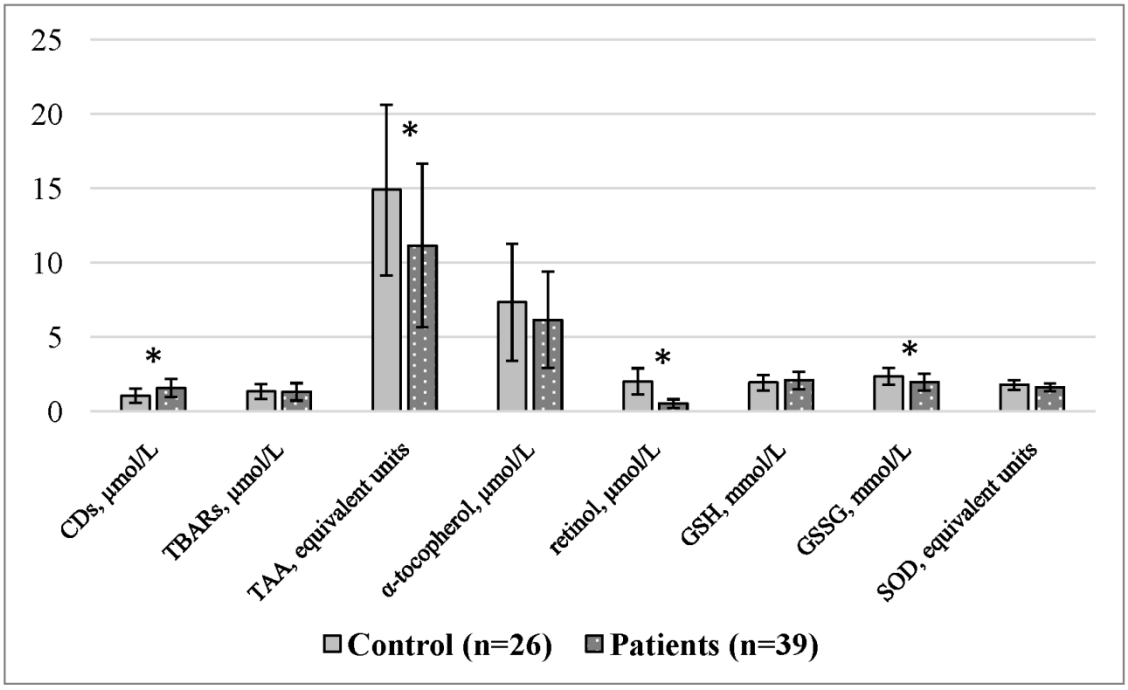

Figure 2. The lipid peroxidation and antioxidant defense systems in girls with constitutional obesity. * statistically significant difference.

To analyze the intra- and intersystem relationships of the control group girls and of those with obesity, a correlation analysis was performed (Table 2). Three connections were established in the control group: TC–LDL (r = 0.86; p = 0.002); LDL–HDL (r = –0.91; p < 0.001); CDs–SOD (r = 0.72; p = 0.019). Eight correlations were established in the group of girls with obesity: TC–VLDL (r = 0.84; p < 0.001); TG–α-tocopherol (r = 0.46; p = 0.014); VLDL–α-tocopherol (r = 0.46; p = 0.014); CDs–GSH (r = –0.38; p = 0.049), TBARs–TAA (r = –0.47; p = 0.011); retinol–TAA (r = –0.42; p = 0.027); retinol–GSSG (r = 0.42; p = 0.028); retinol–α-tocopherol (r = 0.76; p < 0.001).

Table 2. Correlation relationships in girls of control and with constitutional obesity groups*.

Control group	Patients
TC–LDL (r = 0.86)	TC–VLDL (r = 0.84)
LDL–HDL (r = –0.91)	TG–α-tocopherol (r = 0.46)
CDs–SOD (r = 0.72)	VLDL–α-tocopherol (r = 0.46)
	CDs–GSH (r = –0.38)
	TBARs–TAA (r = –0.47)
	Retinol–TAA (r = –0.42)
	Retinol–GSSG (r = 0.42)
	Retinol–α-tocopherol (r = 0.76)

* This table shows only statistically significant correlations (r-correlation value).

4. Discussion

Lipid metabolism analysis in obese girls showed elevated values of total cholesterol, triacylglycerols, and very-low-density lipoproteins. Dyslipidemia is frequently found concomitantly with obesity and is caused, first, by an increase in the consumption of saturated fatty acids [26]. Our data are consistent with the literature, which shows changes in the parameters of adolescent lipid metabolism [6,12,26,27]. A significant factor affecting the dyslipidemia development in obesity is the adipose tissue, especially visceral adipose tissue, which may release free fatty acids that, in excess, enter the portal circulation and the liver [5]. Triglycerides are actively synthesized from free fatty acids in the liver, which is accompanied by an increase in the concentration of VLDL in the blood and a decrease in HDL. Elevated levels of free fatty acids may reduce mRNA expression or lipoprotein lipase activity in adipose tissue and skeletal muscle [28]. An increase in the synthesis of VLDL in the liver can inhibit chylomicron lipolysis, which contributes to hypertriglyceridemia.

Our analysis of the contents of lipid peroxidation products in obese adolescent girls indicates the presence of pro-oxidant reactions at the stage of the primary products. An increase in primary LPO metabolites in obese adolescents suggests the activation of free-radical reactions. Lipid peroxidation products, including TBARs, have a multi-faceted damaging effect on biopolymers and cell structures [17,29]. Lipid peroxidation products are also important as intercellular interaction mediators that implement adaptive mechanisms, so an increase in the concentrations of these parameters may also be a factor in disadaptation in obese patients [30]. Adipose tissue is the largest endocrine organ involved in hormones and cytokines (adipokines) synthesis, accumulation, and metabolism, which, in turn, have endocrine, paracrine, and autocrine effects [31]. Currently, several mechanisms of oxidative stress development in obesity have been established including increased levels of proinflammatory cytokines (e.g., tumor necrosis factor-α, IL-1β, and IL-6), increased free fatty acids as substrates for LPO, and biologically active adipokines [5,12,14,17].

Activation of lipid peroxidation reactions in obesity is also associated with a deficiency in exogenous antioxidants [8]. The antioxidant status of obese girls was characterized by negative changes including significant decreases in TAA, retinol, and GSSG. Inadequate antioxidant defense, along with other factors, such as dyslipidemia, hyperglycemia, chronic inflammation, and hyperleptinemia, contributes to the development of systemic oxidative stress in obesity, and these factors are complementary [14]. The combined insufficiency of a number of antioxidants can contribute to the disruption of the activity of the enzymatic processes and physiological functions that depend on them, as well as adaptive reactions [32]. The parameter TAA reflects the total activity of peroxidation inhibitors and includes numerous enzymatic and non-enzymatic factors, including low-molecular-weight compounds [13]. The inability of the AOD system to function in obese adolescent girls is also confirmed by the reduced concentrations of the fat-soluble vitamin retinol. Retinol is a strong antioxidant that protects membranes from damage caused by superoxide and peroxide radicals [33]. The content of fat-soluble vitamins in children depends not only on their content in food and absorption in the intestine but also on the level of polyunsaturated fatty acids (PUFAs) in food [28]. PUFAs make a significant contribution to the formation of AOD in adolescents. In this case, we can guess about a low supply of retinol and PUFAs in obese adolescent girls, and the main energy consumption can be replenished not at the expense of fat, but at the expense of bread, bakery and grain products. Negative consequences can be caused by the lack of antioxidant vitamins in adolescent girls since the role of antioxidant vitamins as regulators of growth

and morphological differentiation of body tissues is important [34]. These changes may result in a decrease in the resistance of the patients with the dysregulation processes development. In this study, the obese patients showed a decrease in the content of GSSG, which, under pathological conditions, may be associated with compensatory processes, such as an increase in the activity of glutathione reductase. We published similar data previously [35].

The correlation analysis showed the presence of several natural relationships in the control group between the parameters of lipid metabolism. The positive relationship between CDs and SOD enzyme activity may indicate an adequate antioxidant response to the accumulation of LPO products. There was an increase in the number of correlations both within the LPO-AOD system and between the systems in the group of adolescent girls with obesity compared to the control group. The negative dependence of the LPO products and AOD parameters (CDs–GSH, TBARs–TAA, retinol–TAA) indicates insufficient activity of this system. The intersystem dependencies (TG–α-tocopherol, VLDL–α-tocopherol) indicate a significant influence of lipid metabolism changes on the antioxidant defense reactions in obesity.

5. Conclusions

The presence of constitutional obesity in adolescent girls is accompanied by the development of dyslipidemia, as well as by a deficiency in the antioxidant defense components, including total antioxidant activity, retinol, and the oxidized form of glutathione. Together, these data expand our understanding of the pathogenic mechanisms underlying adolescent obesity.

Conflict of interest

All authors declare that there is no conflict of interest in this paper.

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