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# Hypercholesterolemia, Oxidative Stress and Gender Dependence in Children

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**Abstract:** Hypercholesterolemia (HCH) is characterized by an increase of the total- and LDL-cholesterol in serum. In hypercholesterolemia, generally recognized as a risk factor of atherogenesis, oxidative stress and oxidatively modified LDL play a crucial role. In our study, children with elevated total cholesterol (above 4.5 mmol/l) were included. Parameters of lipid profile, lipophilic vitamins and antioxidants (retinol, $\alpha$-tocopherol, $\gamma$-tocopherol, xantophyll, lycophen and $\beta$-carotene) and markers of oxidative damage to lipids (lipoperoxides and 8-isoprostanes) were evaluated. We found that children with hypercholesterolemia have significantly increased parameters of lipid profile and these are gender dependent only in HDL-cholesterol (1.27 ± 0.10 mmol/l in boys vs. 1.53 ± 0.07 mmol/l in girls; $p<0.05$) and TAG (1.63 ± 0.31 mmol/l in boys vs. 1.08 ± 0.09 mmol/l in girls; $p<0.05$). In addition, children with HCH have decreased total antioxidant capacity of serum (TEAC) (about 19.64%, $p<0.05$) and increased lipoperoxides (LP) (about 45.73%, $p<0.001$). We have revealed statistically significant correlations between parameters of lipid profile and lipophilic vitamins and antioxidants, as well as between markers of oxidative stress: positive correlation between LP and 8-iso ($r=0.353$, $n=33$, $p<0.05$) and negative correlations between these parameters and TEAC ($r= –0.377$, $n=33$, $p<0.05$

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for LP and r= –0.379, n=33, p<0.05 for 8-iso). In conclusion, we confirmed relation between hypercholesterolemia and oxidative stress and effect of gender on these processes already in childhood. Since the atherosclerotic process begins in childhood before clinical symptoms, early detection of hypercholesterolemia and oxidative stress is important in later atherosclerosis prevention.

**Introduction**

Hypercholesterolemia is clinically characterized by an increase of the total- and low-density lipoprotein (LDL) cholesterol in plasma. It represents a high risk for the development of atherosclerosis (Pirinccioglu et al., 2010) where also free radicals play a significant role (Steinbrecher et al., 1990), because they are able to oxidatively damage different molecules. One of the most examined free radical damages is an oxidative damage to lipids in the process of lipoperoxidation. Increased lipid peroxidation has been identified as a key mechanism for the development of atherosclerosis by Harrison et al. (2003). According to the original oxidative hypothesis of atherosclerosis, oxidative modification of plasma lipoproteins migrated in the subendothelium exerts pro-inflammatory activities and is the necessary condition for the development of atheroma and its complication (Steinberg et al., 1989). Oxidatively modified LDL play a crucial role in pathogenesis of atherosclerosis (Nagyova et al., 2004; Sumegová et al., 2007). LDL oxidation proceeds in several phases.

In the initiation phase oxidation proceeds very slowly depending on concentration of lipophilic antioxidants in LDL, especially $\alpha$-tocopherol, $\gamma$-tocopehrol and carotenoids (Liptáková, 1999). After depletion of their capacity, the speed of oxidative process markedly increases leading to the formation of lipoperoxides (Esterbauer et al., 1992). Lipoperoxides can be further decomposed and give rise to various secondary products of lipoperoxidation, several biologically active compounds. Some of them have been used as biological markers of lipoperoxidation, like 4-hydroxynonenal (4-HNE) and malondialdehyde (MDA) (Viigimaa et al., 2010). Reaction between MDA/4-HNE and amino groups of proteins leads to a change of proteins. They are recognized by scavenger receptors of macrophages scavenging oxidized LDL and turning themselves into the foam cells. Formation of the foam cells in arterial intima is considered to be the first step in development of atherosclerotic lesions. 4-HNE participates in the development of this disease (Esterbauer et al., 1993; Denke, 1994).

In the past years a new class of biologically effective compounds has been discovered in atherosclerotic lesions. They are called 8-isoprostanes and are products of nonenzymatic peroxidation of arachidonic acid. They serve as potential markers of oxidative stress in atherosclerotic diseases and can modify the function of the vascular wall of the smooth muscle cells and they can accumulate in the vicinity of atherosclerotic plaques (Gniwotta et al., 1997; Patrono and FitzGerald, 1997).

Protective effect of antioxidant vitamins and other lipophilic antioxidants on atherogenetic processes has been known for a long time. One of such vitamins is α-tocopherol. This compound clearly dominates in antioxidant equipment of LDL particles. Recent studies point out that long-term supplementation of vitamin E markedly reduces LDL oxidability in several diseases associated with oxidative stress. Vitamin E in *in vitro* conditions can protect LDL not only against oxidation but also it prevents accumulation of oxidised LDL in macrophages as well as in esters of cholesterol. According to Kwon et al. (2009) vitamin E increases activity of antioxidant enzymes and has a positive effect on prevention against hypercholesterolemia and atherogenesis.

The aim of this study was to determine the lipid profile (total cholesterol (TCH), LDL-cholesterol (LDL-CH), HDL-cholesterol (HDL-CH), total triacylglycerols (TAG), levels of selected lipophilic vitamins and antioxidants (retinol, α- and γ-tocopherol, β-carotene, lycopene and xanthophyll) as well as parameters of oxidative stress (total antioxidant capacity (TEAC), lipid hydroperoxides (LP) and 8-isoprostanes (8-iso)). We were interested in relation between markers of oxidative damage to lipids and parameters of lipid profile, as well as vitamins, antioxidants and TEAC mentioned above.

## Material and Methods

*Patients and sample preparation*

Participants of the study were registered in pediatric center Juvenalia, s.r.o., Dunajská Streda. This protocol was approved by the Ethics Committee of Faculty of Medicine, Comenius University in Bratislava, Slovak Republic. For all participants their parents provided written informed consent before participating in the present study.

To the study 45 healthy children (range 12–13 years) and 38 children (range 11–18 years) with type IIa hypercholesterolemia (ratio TCH/TAG <1.5) were included. Diagnosis of type IIa hypercholesterolemia was based on the mean fasting total serum cholesterol ≥4.5 mmol/l). All children underwent a history and physical examination. The clinical presentation suggests that these children have either familial or polygenic hypercholesterolemia. Only children who had never been treated by hypocholesterolemic drugs were included in the study. None of children were diabetic or hypertensive and none had a history of cardiovascular disease.

Fasting blood samples were taken for analysis. Blood samples were collected from veins into commercial tubes without or with EDTA. After centrifugation (1,200× *g*, 10 min), serum or plasma were obtained, aliquoted and frozen at –80 °C until use.

*Determination of parameters of lipid metabolism*

Serum total cholesterol, HDL-cholesterol and triacylglycerols were analysed by routine biochemical procedures on Hitachi 721 (Roche, Switzerland).

LDL-cholesterol was calculated according to Friedewald's formula (Friedewald et al., 1972) and atherogenic index according to formula: AI = TCH/HDL-CH.

*Determination of lipophilic vitamins and antioxidants*
Lipophilic vitamins and antioxidants were determined by reversed-phase HPLC method according to Hess et al. (1991), which provides measurement of retinol, carotenoids, α-tocopherol and γ-tocopherol in plasma by using fluorescent detector (Watrex, Czech Republic) and detector in the visible range (Watrex, Czech Republic). All results were presented in the μmol/l of serum.

*TEAC (trolox equivalent antioxidant capacity) assay*
The antioxidant capacity of serum was assessed by TEAC assay using 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt tablets (ABTS) (Sigma, USA) according to Re et al. (1999). The method is based on the production of blue-green $ABTS^+$ radical cation formed by potassium persulfate (Lachema, Czech Republic) that was subsequently reduced in the presence of blood antioxidants into colourless form. $ABTS^+$ radical solution was prepared 12–16 hours before each assay. Reaction was initiated by adding of sample to the reaction mixture. The reduction of absorbance was read after 10 minutes and quantification was done on the basis of the dose-response curve for reference antioxidant Trolox, a water-soluble form of vitamin E (Sigma, USA). Results are presented as mmol of Trolox/l.

*Determination of lipid peroxides*
Serum lipid peroxides were assayed spectrophotometrically according to El-Saadani et al. (1989). The analysis was based on the ability of lipid peroxides to convert iodide ($I^-$) to iodine ($I_2$). Subsequently iodine in the reaction mixture reacts with excess of iodide yielding $I_3$ with absorption maximum of 365 nm. All results are presented in nmol/ml of sample.

*Determination of 8-isoprostanes*
To determine the level of 8-isoprostanes, the commercial kit (Cayman Chemical Company, USA) was used. Absorbance was monitored by microplate ELISA reader at the wavelength of 450 nm and level of 8-isoprostanes was expressed in pg/ml.

*Statistical analysis*
All results are presented as the mean ± standard error of the mean (SEM). Student's *t*-test was used for the analysis of data with a Gaussian distribution. P-value of less than 0.05 was considered statistically significant. The Spearman's correlation coefficients were used to determine the relation between parameters. The correlation analysis was performed by using StatsDirect® 2.3.7 (StatsDirect Sales, Sale, Cheshire M33 3UY, UK).

## Results

Impact of gender on basic biochemical parameters reflecting the state of lipid profile in normocholesterolemic (controls) and hypercholesterolemic group (HCH) of children is presented in Table 1. Average values of all monitored parameters in control group were in the range of reference values (RV). After dividing the control group of children (n=45) to the group of boys (n=27) and girls (n=18) no statistically significant differences between groups have been found in lipid parameters.

As expected, children with hypercholesterolemia exhibited a significantly higher level of total cholesterol, LDL-cholesterol, TAG, as well as atherogenic index compared to control group. In the study significantly higher level of HDL-cholesterol in the HCH group has been found, but this has not influenced the atherogenic index.

After dividing of hypercholesterolemic children according to gender, significantly increased levels of HDL-CH and decreased levels of TAG ($p<0.05$) have been found in girls compared to boys.

**Table 1 – Parameters of lipid profile in normo- and hypercholesterolemic children**

| Gender/ Parameter | TCH (mmol/l) | LDL (mmol/l) | HDL (mmol/l) | TAG (mmol/l) | AI TCH/HDL-CH | BMI (kg/m$^2$) |
|---|---|---|---|---|---|---|
| | | | Control groups | | | |
| All | 3.74±0.05 (45) | 2.23±0.05 (45) | 1.28±0.04 (41) | 0.96±0.10 (45) | 3.04±0.10 (41) | 19.99±0.82 (45) |
| Boys | 3.73±0.08 (27) | 2.25±0.06 (27) | 1.25±0.05 (24) | 0.96±0.10 (27) | 3.12±0.15 (24) | 20.27±1.02 (26) |
| Girls | 3.76±0.06 (18) | 2.20±0.08 (18) | 1.32±0.06 (17) | 1.03±0.19 (18) | 2.93±0.14 (17) | 19.59±1.42 (18) |
| pB/G | ns | ns | ns | ns | ns | ns |
| | | | HCH groups | | | |
| All | 5.02±0.13 (38) | 3.12±0.12 (38) | 1.44±0.06 (38) | 1.25±0.12 (38) | 3.69±0.18 (38) | 20.89±0.79 (38) |
| Boys | 4.77±0.26 (12) | 2.96±0.24 (12) | 1.27±0.10 (12) | 1.63±0.31 (12) | 4.05±0.40 (12) | 23.67±1.21 (10) |
| Girls | 5.13±0.14 (26) | 3.20±0.13 (26) | 1.53±0.07 (26) | 1.08±0.09 (26) | 3.53±0.18 (26) | 20.20±0.85 (23) |
| pB/G | ns | ns | p<0.05 | p<0.05 | ns | p<0.05 |
| pHCH/C | p<0.001 | p<0.001 | p<0.05 | p<0.05 | p<0.01 | ns |
| RV | <4.5 | <3.4 | <1.6(G) <1.42(B) | <2.3 | | |

Data are given as mean ± SEM (n); n – number of subjects per group; G – girls; B – boys; HCH – hypercholesterolemic children; RV – reference values; pB/G – statistically significant difference between boys and girls; pHCH/C – statistically significant difference between hypercholesterolemic and normocholesterolemic (control) groups; ns – nonsignificant difference

Lipophilic antioxidants have an important role in protection of lipoproteins against oxidation. Results of the present study have revealed that the levels of retinol, α-tocopherol, γ-tocopherol, lycopene and β-carotene are in the reference range (according to: Ford et al., 2002; Krajcovicova-Kudlackova et al., 2009) in children with hypercholesterolemia and they were similar in the plasma of both genders (Table 2). Although levels of these lipophilic compounds have not been significantly different between genders in HCH group, we have found that in the group of boys retinol level positively correlates with the total cholesterol ($r=0.500$, $n=12$, $p<0.05$), LDL-CH ($r=0.553$, $n=12$, $p<0.05$) and triacylglycerols ($r=0.610$, $n=12$, $p<0.05$) and negatively correlates with HDL-CH ($r= -0.687$, $n=12$, $p<0.05$). These correlations have not been observed in the group of girls. However, in

**Table 2 – Parameters of vitamins in children with hypercholesterolemia**

| Gender/ Parameter | Retinol (μmol/l) | Xanthophyll (μmol/l) | α-tocopherol (μmol/l) | γ-tocopherol (μmol/l) | Lycopene (μmol/l) | β-carotene (μmol/l) |
|---|---|---|---|---|---|---|
| | | | HCH groups | | | |
| All | 3.27±0.11 (38) | 0.15±0.01 (38) | 1.16±0.07 (38) | 26.02±1.05 (38) | 0.40±0.03 (38) | 1.02±0.17 (38) |
| Boys | 3.35±0.11 (12) | 0.15±0.02 (12) | 1.26±0.13 (12) | 26.41±1.63 (12) | 0.38±0.04 (12) | 0.68±0.24 (12) |
| Girls | 3.10±0.16 (26) | 0.16±0.01 (26) | 1.12±0.09 (26) | 25.84±1.36 (26) | 0.40±0.04 (26) | 1.17±0.24 (26) |
| pB/G | ns | ns | ns | ns | ns | ns |

Data are given as mean ± SEM (n); n – number of subjects per group; G – girls; B – boys;
HCH – hypercholesterolemic children; pB/G – statistically significant difference between boys and girls

**Table 3 – Parameters of oxidative stress in normo- and hypercholesterolemic children**

| | Control groups | | | HCH groups | | |
|---|---|---|---|---|---|---|
| Gender/ Parameter | TEAC (mmol/l) | LP (nmol/l) | 8-iso (pg/ml) | TEAC (mmol/l) | LP (nmol/l) | 8-iso (pg/ml) |
| All | 1.68±0.10 (40) | 32.60±2.70 (40) | na | 1.35±0.10 (38) | 47.51±2.03 (38) | 87.82±7.99 (34) |
| Boys | 1.53±0.11 (23) | 34.67±4.13 (24) | na | 1.41±0.20 (12) | 49.32±2.03 (12) | 91.04±14.05 (11) |
| Girls | 1.89±0.19 (17) | 29.49±3.61 (16) | na | 1.28±0.12 (25) | 46.68±2.03 (26) | 86.29±9.71 (23) |
| pB/G | $p<0.05$ | ns | na | ns | ns | ns |
| pHCH/C | | | | $p<0.05$ | $p<0.001$ | na |

Data are given as mean ± SEM (n); n – number of subjects per group; G – girls; B – boys; HCH – hypercholesterolemic children; na – nonanalysed samples; pB/G – statistically significant difference between boys and girls; pHCH/C – statistically significant difference between hypercholesterolemic and normocholesterolemic (control) groups; ns – nonsignificant difference

the group of girls positive correlations of compounds with lipophilic antioxidant properties like xanthophyll ($r=0.316$, $n=26$, $p<0.05$), $\alpha$-tocopherol ($r=0.465$, $n=26$, $p<0.05$), $\gamma$-tocopherol ($r=0.568$, $n=26$, $p<0.05$) and lycopene ($r=0.405$, $n=26$, $p<0.05$) with HDL-CH have been found.

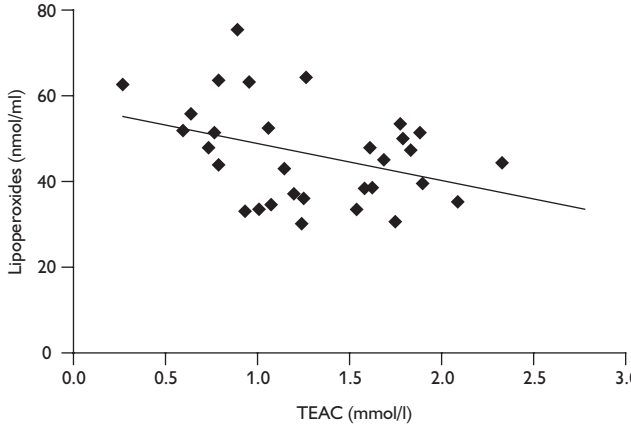


*Figure 1 – Relation between lipid hydroperoxides and the total antioxidant capacity of plasma (TEAC) in hypercholesterolemic group ($r= –0.377$, $n=33$, $p<0.05$).*

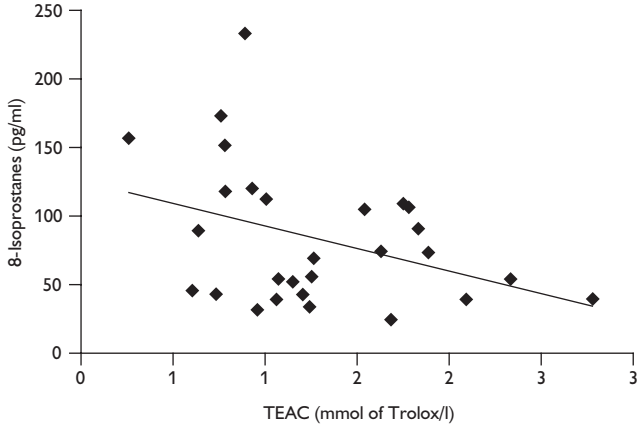


*Figure 2 – Relation between 8-isoprostanes and the total antioxidant capacity (TEAC) in hypercholesterolemic group ($r= –0.379$, $n=33$, $p<0.05$).*

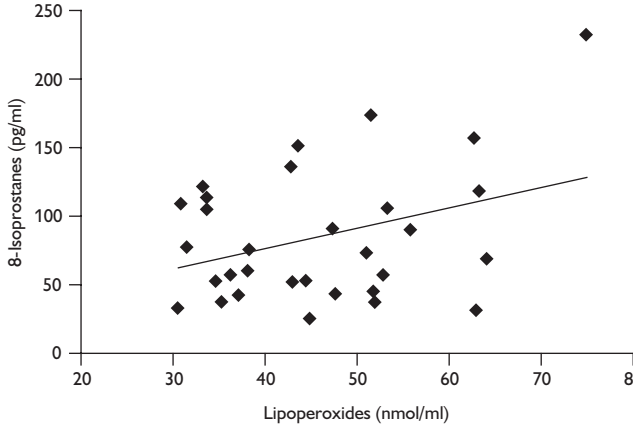


*Figure 3 – Relation between lipid hydroperoxides and 8-isoprostanes in hypercholesterolemic group ($r=0.353$, $n=33$, $p<0.05$).*

As shown in Table 3, when compared to normocholesterolemics, a significantly higher value of serum lipid hydroperoxides and significantly lower value of total antioxidant status (by 45.73% and 19.64%, resp.) has been observed in the hypercholesterolemic children.

The total antioxidant status of serum was significantly lower in normocholesterolemic boys than in girls (by 19.04%). No statistically significant differences between boys and girls have been found in groups of children with hypercholesterolemia.

The level of lipoperoxides has not been gender dependent in both, hypercholesterolemic and control groups. Even if no relation between lipid hydroperoxides and parameters of lipid profile in our experimental group of children with hypercholesterolemia have been observed, in the group of boys we have found the positive correlations between TCH, resp. LDL-CH and level of lipoperoxides ($r=0.474$, resp. $r=0.545$, $n=12$, $p<0.05$).

Between individual parameters of oxidative stress some associations have been revealed. Figures 1–3 present relation between lipid hydroperoxides, 8-isoprostanes and TEAC. Lipid hydroperoxides as well as 8-isoprostanes negatively correlate with TEAC ($r= -0.377$, resp. $r= -0.379$, $n=33$, $p<0.05$) and lipid hydroperoxides positively correlate with 8-isoprostanes ($r=0.353$, $n=33$, $p<0.05$) in group of HCH children.

**Discussion**

Atherosclerosis belongs to serious inflammatory diseases developing from the childhood and it is clinically manifested in the middle or older age. In the Slovak Republic an alarming fact has been found that in average 25% children have elevated cholesterol levels compared to reference values. Oxidative modification of lipoprotein particles is one of the basic causes of initiation and progression of atherosclerotic changes in the vascular endothelium.

This status named hypercholesterolemia is clinically characterized by an increase of the total- and low-density lipoprotein (LDL) cholesterol in plasma. Increased total cholesterol (TCH) seems to be a major determinant of early atherosclerosis. Several longitudinal studies have confirmed that the number of cardiovascular risk factors present in adolescence, including elevated cholesterol, is also relevant in the progression of atherosclerosis in adults and indicates a higher risk of heart diseases or heart attacks (Gooding and de Ferranti, 2010). Thus, measurement of LDL in childhood predicts carotid intima-media thickness in young adults (Martino et al., 2008). On that account, screening and subsequent therapy of hypercholesterolemia has importance in the prevention of future cardiovascular diseases.

Optimal value of the total cholesterol in children should not exceed 4.5 mmol/l (Nagyova et al., 2004; Sumegová et al., 2007). In our experimental group we have found all lipid parameters (total cholesterol, LDL-CH, HDL-CH, triacylglycerols) elevated in comparison to control group of clinically healthy children. Although

HDL-CH has not been decreased, atherogenic index in HCH group has been higher and gender dependent. According to our results boys exhibit higher risk of atherosclerosis that girls. This difference could be explained by higher BMI in boys compared to girls, what is further of cardiovascular risk factors. Positive correlation between BMI and TAG, which have been found in our experimental group, warns against children's overweight accompanied by hypercholesterolemia and confirms the fact that children's overweight and impairment in lipid metabolism represented by increased total cholesterol level and TAG are dangerous risk factors for increased oxidative damage and for later development of atherosclerosis.

Gender differences in the pathogenesis, progression and manifestation of large variety of cardio- and cerebrovascular diseases have been well documented (Ren, 2007).

In our previous studies we also have confirmed the effect of genders on atherogenic risk. In adult women the increased HDL-CH and decreased LDL-CH and TAG were found by Sumegová et al. (2006b), what confirms our results found in children. The same group (Sumegová et al., 2006a) found increased oxidized LDL and simultaneously, significant increased lipoperoxides levels in adult men in comparison to women. These findings could be explained by higher arylesterase activity of paraoxonase, the antioxidant enzyme associated to HDL, which prevent lipoproteins against lipoperoxidation (Sumegová et al., 2007). In addition, it is well known that lipid profile as well as oxidative damage of lipids depend from ovarial hormones, especially estrogen and progesterone (Ren, 2007), what is related to protection of females against atherosclerosis. In our study 58% of girls after the first period were included, what also could explain gender differences.

Important role in process of atherosclerosis plays oxidative stress, especially oxidative modification of LDL cholesterol. Oxidative stress is an imbalance between pro-oxidatively acting compounds and antioxidants in favour of pro-oxidants causing the damage of important biomolecules (Ďuračková, 1998). It can directly evoke development of a disease or worsen or complication its progress. Oxidative stress participates in pathogenesis of atherogenesis through oxidative damage to lipids in the process of lipoperoxidation. The initiation phase of lipoperoxidation proceeds very slowly depending on concentration of lipophilic antioxidants in LDL, especially $\alpha$-tocopherol, $\gamma$-tocopherol and carotenoids (Liptáková, 1999), which protect cells against damage induced by oxidative stress (Al-Azemi et al., 2009). We were interested in level of lipophilic vitamins and antioxidants in HCH children. We have not found any differences in comparison to reference values, valid for our laboratory.

Correlations between parameters of lipid profile and lipophilic vitamins and antioxidants (retinol, $\alpha$-tocopherol a $\gamma$-tocopherol) indicate that the more lipoproteins in blood plasma afford the more lipophilic milieu for fat-soluble vitamins. Importance of lipophilic milieu confirmed Muldoon et al. (1996) who revealed, that hypocholesterolemia is associated with reduced antioxidant reserve.

Low cholesterol was associated with reduced vitamin E level, possibly increasing susceptibility to oxidative stress.

Based on our results we can conclude gender differences in the abundance of lipophilic vitamins and antioxidants in the lipoproteins. In the group of hypercholesterolemic boys the level of retinol positively correlated with TCH, LDL-CH and TAG, whereas in the group of girls positive correlations between HDL-CH and α- and γ-tocopherols, as well as with xanthophyll and lycopen have been found.

Our results are in concord with results of Tonstad and Aksnes (1997), who determined retinol and α-tocopherol in the boys and girls with hypercholesterolemia. They revealed, that TAG level was positively related to level of retinol and concluded, that lipid levels and gender are significant predictor of fat-soluble vitamins level in these children.

Al-Azemi et al. (2009) observed that serum retinol and α-tocopherol in women were significantly higher than in men. There was an inverse relation between BMI and serum retinol resp. α-tocopherol in both men and women. This relation has not been found in our experimental group.

We have not found alteration in lipophilic vitamins and antioxidants in the group of HCH children. Despite of this we have observed statistically significant elevation of lipid hydroperoxides (marker of oxidative damage to lipids) and reduction of the total antioxidant serum capacity. The exhaustion of hydrophilic antioxidants measured by TEAC method could explain increased oxidation to lipids, what confirms cooperation between hydrophilic and lipophilic antioxidants in serum.

Minuz et al. (2006) determined increased level of plasma 8-isoprostanes and increased excretion of urinary 8-isoprostanes in the subject with cardiovascular risk factors compared to control subjects. Similarly, Vassalle et al. (2003, 2004) and Martino et al. (2008) found elevated levels of plasma 8-isoprostanes and reduced antioxidant capacity. Their findings indicate association of these parameters with the extent and the severity of coronary artery disease and with the occurrence of different atherogenic risk factors. In addition, early and persistent oxidative stress in HCH children was confirmed also by Pignatelli et al. (2009).

These results do not correspond to results of Cracowski et al. (2001), who concluded, that 8-isoprostane level in children with type IIa hypercholesterolemia does not differ from those of age- and sex-matched control children, what means, that hypercholesterolemia is not associated with an increased lipid peroxidation in childhood.

However, our findings of increased level of lipoperoxides in HCH group, as well as assignment of negative correlations between lipoperoxides, resp. 8-isoprostanes and TEAC confirmed participation of oxidative stress in hypercholesterolemic children. Positive correlations between TCH, resp. LDL-CH and level of lipoperoxides in the group of boys establish our previous results too.

To limitations of our work belong unequal number of participants in the group of boys and girls in control and HCH groups, as well as disability to obtain results of lipophilic vitamins, antioxidants and 8-isoprostanes in control group from technical point of view.

To conclude we confirmed relation between hypercholesterolemia and oxidative stress and effect of gender on these processes already in childhood. Since the atherosclerotic process begins in childhood before clinical symptoms, early detection of hypercholesterolemia and elimination of oxidative stress in children for example through modulation of life-style and diet may be important for reduction of risk of later atherosclerosis in adults.

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