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## Original Research Article

# Assessment of changes in lipid profile and related enzymes in children with asthma

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### Abstract

**Purpose:** To investigate the influence of the lipid profile and related parameters on the development of asthma in children aged 10 to 15 years.

**Methods:** Peripheral blood samples were collected from a group diagnosed with asthma as well as from a healthy control group. The lipid profile parameters measured were total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL), high-density lipoprotein (HDL), serum total antioxidant capacity (TAC), reduced glutathione (GSH), and malondialdehyde (MDA), and the activities of lecithin-cholesterol acyltransferase (LCAT) and cholesteryl ester transfer protein (CETP).

**Results:** TC, TG, LDL, and VLDL levels were significantly ( $p \leq 0.05$ ) higher in the asthma group compared with the controls, while HDL level was lower. Total TAC and GSH were lower in the asthma group, while MDA level, and LCAT and CETP activities were higher.

**Conclusion:** There is a link between an elevated lipid profile and increased antioxidant capacity in asthmatic children.

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## INTRODUCTION

Asthma is a heterogeneous disease that affects both children and adults, and is characterized by chronic inflammation in the respiratory tract due to continuous inflammatory stress signaling or chronic exposure to allergens [1]. Symptoms of asthma include wheezing, shortness of breath, tightness in the chest, and cough of varying intensity [1]. Asthma is a chronic health problem that affects 20 % of children worldwide [2]. Globally, asthma-related problems have increased by 30 % over the past 20 years [3].

Measurement of the nitric oxide concentration in exhaled breath is a non-invasive method of monitoring the status of airway inflammation due to asthma in children older than 1 year [4]. Any infection in the upper respiratory tract with recurrent coughing and wheezing, followed by an elevated concentration of exhaled nitric oxide for more than 4 weeks, is taken as a clinical marker of asthma in children [5,6].

Reports suggest a strong correlation between oxidative stress and lung

diseases, including asthma, chronic obstructive pulmonary disorder, cystic fibrosis, and lung cancer [7]. In the body, oxidative stress is mitigated by the antioxidant system, which consists of enzymatic and non-enzymatic antioxidants [8]. Failure of the antioxidant defense system can result in oxidant-mediated injury, leading to cell death [8]. Acute asthma exacerbations may lead to increased oxidative stress, which in turn causes reduced superoxide dismutase (SOD) activity [9]. It has been reported that extracellular glutathione (GSH) peroxidase activity is increased in the bronchial epithelial cells of asthmatic subjects [10,11]. Superoxide and hydrogen peroxide radicals also increase vascular endothelial growth factor expression, which can be blocked by antioxidants [12].

Advances in the diagnosis of asthma have led to both under- and over-diagnosis of the disease [2]. Under-diagnosis could lead to an increased burden and higher healthcare costs, while over-diagnosis could lead to higher treatment costs and exposure to unwanted side-effects. Globally, nearly one-third of all children and adolescents are classified as either overweight or obese and show abnormal lipid profiles [13]. Therefore, it is very important to identify correlations among the clinical parameters and markers that can help to diagnose asthma accurately. This study examined the correlations between asthma in children and the lipid profile and related blood parameters, with the aim being to identify factors that favor the initiation and development of asthma.

## METHODS

### Study design and subjects

Children between 10 and 15 years of age, and presenting to Jining No. 1 People's Hospital, Shandong, China for

asthma treatment, were initially screened for symptoms of asthma using a standard protocol. Children diagnosed with asthma were selected randomly for inclusion in the study. The study objectives, experimental design, and protocol were explained to the subjects in the presence of their parents or legal guardians, from whom written consent was obtained. Age, sex, height (m), and weight (kg) data were collected. The body mass index (BMI) of the subjects was calculated using the standard formula ( $\text{kg}/\text{m}^2$ ). The study matched 59 children (28 boys, 31 girls) in the asthma group by age and gender with 57 (29 boys, 28 girls) children in the control group. After a minimum 10-h fast, 7 mL of venous blood was collected from each subject. Serum was prepared from the blood samples in a refrigerated table-top centrifuge and stored at  $-80\text{ }^\circ\text{C}$  until analysis. The experiment was approved by the Ethical Review Committee of the Jining No.1 People's Hospital (approval Ref no 1603312) complied with international guidelines [14].

### Determination of lipid profile parameters

The total cholesterol (TC), high-density lipoprotein (HDL) cholesterol and triglycerides (TG) were assayed using commercial kits (BioSino Bio-technology and Science, Shanghai, China), following the manufacturer's instructions. Low-density lipoprotein (LDL) cholesterol was calculated from the measured TC, TG, and HDL cholesterol using the following formula:  $\text{LDL-cholesterol} = \text{total-cholesterol} - \text{HDL-cholesterol} - [\text{TG}/5]$ , where  $[\text{TG}/5]$  is an estimate of very-low-density lipoprotein (VLDL) cholesterol; all values are in mg/dL [15].

### CETP and LCAT assays

The CETP and lecithin-cholesterol acyltransferase (LCAT) activities were assayed in serum using commercial fluorometric assay kits (Roar Biomedical,

New York, NY, USA). Buffers and reagents were used as provided, and the standard assay protocol followed the manufacturer's instructions. CETP Activity Assay Kit uses a proprietary substrate that enables the detection of CETP-mediated transfer of neutral lipid from the substrate to a physiological acceptor. The transfer activity results in an increase in fluorescence intensity. LCAT activity was obtained by a fluorometric assay that measures the phospholipase activity of LCAT.

#### Determination of the total antioxidant capacity of serum

The total antioxidant capacity (TAC) of the serum was measured based on a colorimetric method developed by Erel [16]. The assay mixture consists of ferrous ion solution and hydrogen peroxide, which produce hydroxyl radicals that generate brown dianisidiny radical cations. The antioxidant capacity of the serum was measured against the potent free radical reactions initiated by the hydroxyl radicals produced. The assay results are expressed in mM of Trolox equivalents/L.

#### Evaluation of serum reduced glutathione

Reduced GSH in serum was analyzed using the method of Ellman *et al* [17] and expressed in mg/dL. This enzymatic recycling method uses GSH reductase to quantify GSH. The sulfhydryl group of GSH reacts with 5,5'-dithio-bis-2-nitrobenzoic acid (Ellman's reagent) and produces yellow 5-thio-2-nitrobenzoic acid (TNB). Oxidized glutathione (GSSG) is then reduced to GSH by GSH reductase, using reducing equivalents provided by NADPH. The rate of TNB formation is proportional to the sum of GSH and GSSG present in the sample, determined by measuring the formation of TNB at 412 nm.

#### Serum malondialdehyde levels

Malondialdehyde (MDA) in serum was analyzed using the method of Ohkawa *et al* [18]. Briefly, serum was reacted with trichloroacetic acid containing 2-thiobarbituric acid, and the mixture was incubated at 95 °C for 20 min and then cooled in ice. The thiobarbituric acid content was measured as the MDA ( $\epsilon = 155 \text{ mM}^{-1}\text{cm}^{-1}$ ) content at  $A_{532}$  using a spectrophotometer and expressed as nM/L.

#### Statistical analysis

All group data are presented as mean  $\pm$  SD. Significant differences between the groups were analyzed using one-way analysis of variance followed by Tukey's honestly significant difference *post-hoc* test. Statistical significance was set at  $p \leq 0.05$ .

## RESULTS

Table 1 summarizes the subjects' characteristics. The mean age did not differ significantly between the two groups, so there was no age-related bias. BMI was markedly higher in the asthma group.

**Table 1:** Characteristics of subjects in the asthma and control groups

Parameter	Asthma	Control
Age (years)	12.5 $\pm$ 2.3	12.8 $\pm$ 2.5
Sex (M:F)	28:31	29:28
Weight (kg)	32.3 $\pm$ 4.7	28.6 $\pm$ 3.8
BMI (kg/m <sup>2</sup> )	25.6 $\pm$ 3.2	20.4 $\pm$ 2.7

BMI = body mass index

TC, TG, LDL, and VLDL levels were significantly increased in the asthma group (30.12, 24.17, 28.66, and 17.15 % higher than those of the controls, respectively; Table 2). As expected, there was a corresponding decrease in HDL level in the asthma group, which was 15.08 % lower than that of the controls. CETP activity was 13.54 % higher in the asthma versus control group, and the difference was significant. LCAT activity was 9.4 % higher in the asthma than in the control group, but the difference was not significant.

TAC was decreased by 33.33 % in the asthma group relative to the controls, while the serum MDA was increased by 47.59 %, and the serum GSH was decreased by 12.09 %.

Malondialdehyde (MDA) level was significantly higher ( $p \leq 0.05$ ) in asthma group. On the other hand, glutathione (GSH) levels was significantly lower in the asthma compared to the control groups.

**Table 2:** Lipid profiles of the asthma and control groups. Values are means  $\pm$  SD (units: mg/dL)

Parameter	Asthma	Controls
TC	189.63 $\pm$ 16.56*	145.74 $\pm$ 16.71
HDL	42.39 $\pm$ 5.42*	49.92 $\pm$ 3.94
TG	133.49 $\pm$ 15.98*	107.51 $\pm$ 11.38
VLDL	26.23 $\pm$ 2.65*	22.39 $\pm$ 2.14
LDL	108.55 $\pm$ 9.38*	84.37 $\pm$ 10.27

TC, total cholesterol; HDL, high-density lipoprotein; TG, triglycerides; VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein; \* $p \leq 0.05$

## DISCUSSION

Higher mitochondrial cholesterol levels can lead to impaired GSH transport across the mitochondrial membrane, inhibiting the removal of free radicals generated in mitochondria and the excessive accumulation of reactive oxygen species (ROS) [11]. This could result in mitochondrial DNA damage, loss of essential mitochondrial protein synthesis, and progressive respiratory dysfunction, in turn leading to many diseases [8]. In our study, the significantly elevated cholesterol and LDL and VLDL levels are clues to the role of the lipid profile and increased free radical generation in asthma.

Ectopic expression of the  $\alpha$ - and  $\beta$ -chains of ATP synthase in liver cells is essential for HDL catabolism [19]; disturbance in this expression could lead to disease. The low serum HDL cholesterol levels observed in the children in our study could be the reason for the initiation or development of asthma.

Invasive and non-invasive interventions have been developed to increase HDL

cholesterol, and decrease LDL cholesterol, in blood and to promote health.

Cholesteryl ester transfer protein (CETP) and lecithin cholesterol acyl transferase (LCAT) are enzymes involved in HDL remodeling and metabolism [20]. CETP facilitates the exchange of TG with cholesteryl ester, or vice versa, between HDL and other lipoproteins, ultimately leading to lower HDL, and higher LDL and VLDL, levels [21]. The increased CETP activity in the asthma group in our study explains the occurrence of metabolic reactions that favor the production of lipoproteins other than HDL, which might in turn explain the elevated LDL and VLDL levels, and decreased HDL level, among the asthmatic children.

TG is a vital molecule for determining the HDL level in blood. An increased blood TG level could cause the addition of TG molecules to HDL, leading to hydrolysis by hepatic lipase and a reduction in the blood HDL level [22]. LCAT esterifies cholesterol via an acyl group, derived preferably from HDL molecules [23]. Abnormal lipid molecules have been observed in the absence of LCAT activity [23]. In our study, the LCAT activity was increased slightly, albeit not significantly, in the asthma group.

Free radicals, which generate ROS, are produced via electron transport in mitochondria, and by NADPH oxidase activity in neutrophils, monocytes and macrophages, molybdenum hydroxylase, and arachidonic acid metabolism, which reacts with metal prosthetic groups or iron-sulfur centers in proteins, causing loss of functionality [11,24].

Various cell types initiate the antioxidant defense process via enzymatic and non-enzymatic antioxidants [11]. In a biological system, the ratio between the

inter-convertible oxidized and reduced forms of a specific protein or molecular pair is used to evaluate the redox environment and its reducing capacity [24]. In the present study, the significantly lower TAC and GSH levels seen in asthmatic patients versus controls support previous reports of increased MDA levels. GSH may be present in high concentrations in blood and the lungs.

Lung epithelium contains 300 micromoles of GSH, more than 90 % of which is in reduced form [25]. The importation of reduced GSH into cells, and exportation of oxidized GSH, could contribute to a reduction in oxidative stress [12]. In the present study, the lower GSH levels in the blood of the asthma subjects might have been insufficient for transport into the lungs, which could lead to the initiation and development of asthma. The data support the hypothesis that ROS and reactive nitrogen species (RNS) are responsible for the inflammation (via the the accumulation of inflammatory cells), airway obstruction, and hyper-responsiveness seen in asthma [26], while ROS-mediated modifications in oxidation have been linked with asthma severity [27].

As our subjects were young, and thus had a high basal metabolic rate and level of physical activity, their tidal volume might also be high. An abundance of oxygen in the lungs could be responsible for adding more ROS and RNS to the reactive radicals produced metabolically [11]. This could worsen asthma still further, if not balanced by the removal of radicals via interventions based on nutritional supplements and detoxifiers.

### Study limitations

Lipid-related complications might pre-initiate disease, which could lead to

asthma and many other related disorders. Although a clear connection between antioxidative capacity and asthma in obese children has been established, more work is needed to establish these parameters as biomarkers.

### CONCLUSION

We established clear connections among asthma, the lipid profile, and antioxidant defense in obese children. The TC, TG, LDL, and VLDL levels, and LCAT and CETP activities, were significantly higher in our asthma versus control group. The asthmatic patients also suffered from oxidative stress and subsequent inflammation.

### DECLARATIONS

#### *Acknowledgement*

None declared.

#### *Conflict of Interest*

No conflict of interest associated with this work.

#### *Contribution of Authors*

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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