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Short communication

Distribution of the total unsaturation in lipid components of plasma as a new differential diagnostic method in clinical analysis

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Abstract

Using ozonization and thin-layer chromatographic methods we determined the qualitative and quantitative correlation of unsaturation distribution (UD) in individual fractions of blood plasma lipids in children suffering from insulin-dependent *diabetes mellitus* (IDDM). The research was aimed at elucidation of biochemical criterion of the degree of metabolic disorders in children with IDDM and at development of methods for quantitative assessment of such disorders. Twenty children were examined during the compensation stage (group 1), and twelve during decompensation with ketoacidosis (group 2). The present investigation shows that in the case of insulin-dependent *diabetes mellitus* in children the total unsaturation distribution (TUD) in plasma lipid fractions were found to be decreased significantly compared to healthy controls. The pattern of TUD in plasma lipid fractions may serve as a new biochemical criterion for metabolic disorders and decompensation in IDDM. © 1997 Elsevier Science B.V.

Keywords: *Diabetes mellitus*; Lipids

1. Introduction

Advanced chromatographic methods are finding expanding application in medical research and clinical practice. They make it possible to estimate disturbances in metabolism for a variety of diseases. Special attention is paid to the investigation of the blood plasma lipid structure of patients for diagnostic purposes and in clinical pathology [1–5].

In numerous studies directed toward investigation of *diabetes mellitus* (DM) pathogenesis, great attention is paid to lipid metabolism disorders [6–9]. Recently, the important role of unsaturated fatty acids (USFAs) in the formation of highly active compounds was shown, including arachidonic acid, prostaglandins predecessor, which is of special interest, as well as linoleic and linolenic acids that are mobilized in the first place in the cases of insulin dependent *diabetes mellitus* (IDDM). It is also well known that in the DM fatty acid profile and the USFA concentration are altered in plasma, and these changes impact both free fatty acids and lipid complex acids [7,10,11]. Correlation was shown between state hardness in DM patients with character and extent of USFA spectrum and concentration level [7]. It was observed that these factors are under the influence of treatment measures. However, fatty acid profile determination by chromatographic methods is not suitable for monitoring of the patients

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condition because of the time-consuming complex analysis [8].

The estimation of the total unsaturation (TU) of plasma (DB index) lipids is a new and promising method for testing various pathologies [12–16]. This method consists of measuring the ozone absorbed by lipid double bonds according to the ozonization reaction [17]. The quantity of absorbed ozone is the measure of the TU of plasma lipids (DB index) [14,15].

In the present work children suffering from IDDM were examined for TU of blood plasma lipids and for unsaturation in individual fractions of blood plasma lipids. The objective of the present work was to find a new biochemical criterion for metabolic disorders and decompensation in IDDM and to develop the methods for quantitative assessment of such disorders.

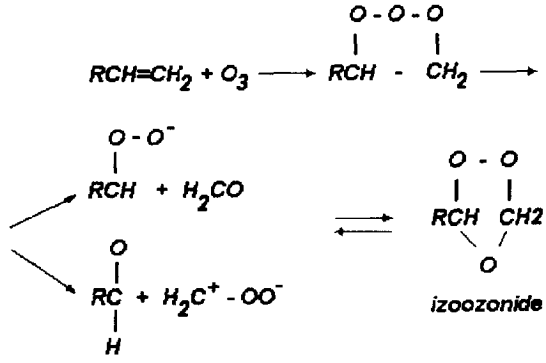
2. Experimental

2.1. Sample preparation

Samples for measurement were prepared by extracting according to a simplified Folch method [18]. For lipid extraction without protein stage filtration and solution evaporation 0.4 ml of chloroform and 0.2 ml of methanol were successively added to 0.05 ml of blood plasma. Then 0.25 ml of distilled water was added and the sample was maintained at T=4°C for 10 min. A 10 μl lipid solution in chloroform was injected into the "Double Bonds Analyzer" (DBA) reactor.

2.2. Methods and instrumentation

Lipid fractionation was carried out by thin-layer chromatography (TLC) on the "Silufol" plate with next registration on the EPI-65 densitometer (Carl Zeis, Jena, Germany). The blood plasma lipid profile was analyzed for the following fractions: phospholipids (PhLs), free cholesterol (FCh), non-etherificated fatty acids (NEFAs), triglycerides (TGs) and cholesterol ethers (ChEs). The total unsaturation of the lipid fractions was carried out by the ozonization method [19,20]. This method consists of measuring the ozone absorbed by lipids double bonds according to the following ozonization reaction [17]:



The quantity of absorbed ozone is the measure of the total unsaturation of blood plasma lipids (DB index) [13–16]. The ozone absorbed by lipids double bonds was determined spectrophotometrically in gaseous mixture in the reactor outlet. This technique is carried out with the help of the compact original device "Double Bonds Analyzer" [21], which has an ozone working concentration from 10^{-6} to 10^{-8} mol/l, with precision up to 1%, selectivity 10^{8} and with a short analysis time from 2 s to 30 s per test.

The total unsaturation distribution (TUD) in plasma lipid fractions was determined by the solid state ozonization method of the "Silufol" powders with the lipid fraction absorbed on principle of "fluid bed" (with particle size less 0.1–0.2 μm) at T= 20°C. The minimum determined concentration of lipid double bonds in that case amounts to 10^{-6} mol d.b./g. The standard error was estimated about 4%. The TU of the liquid and powder samples was calculated by "standard-sample" method using the formula [14] for the liquid samples:

$$\mathrm{DB_{liq}\ index} = \frac{c_{st} V_{st} s_s V_{sol}}{s_{st} V_s V_{pl} K}\ \text{(c.u.)} \qquad (1)$$

where c_{st} is concentration of the standard solution (mol/ml), V_{st}, V_s are the volumes of the standard sample and the analyzed sample, respectively (ml), s_{st}, s_s are the areas of the standard ozonograms and the analyzed sample (c.u.), V_{sol} is the solution volume of the analyzed sample (ml), V_{pl} is the blood plasma volume of the analyzed sample (ml), K is the calculated coefficient equal to 10^{-7} (ml/mol), (c.u.)

Table 1
The total unsaturation distribution in plasma lipid fractions in children groups of different stage

Children groups	DB_{liq} index c.u.	DB_{pow} index, rel%				
		PhL	FCh	NEFA	TG	CHF
Control	260.0±10	13.9±5.0	10.0±2.3	43.4±7.1	17.5±1.9	15.0±3.1
1st	164.3±11[a]	13.8±3.2	14.3±1.9	40.5±7.4	14.6±1.9	16.8±2.1
2nd	150.5±11[a]	17.1±2.8	14.7±1.9	23.7±6.1[b]	22.5±1.2[b]	22.1±1.9

[a] $P \leq 0.001$.
[b] $P \leq 0.05$.

means "conditional unit" (1 c.u. $= 1 \times 10^{-5}$ mol d.b./ml).

The TU of the powder samples was calculated by the formula [13]:

$$DB_{pow}\ \text{index} = \frac{c_{st} V_{st} s_s}{s_{st} G}\ (\text{mol d.b./g}) \qquad (2)$$

where G is hanging of the lipid fractions (g).

2.3. Patients

We examined 164 children aged from 1–14 years with IDDM. The control group was composed of twenty healthy children of the same age. The patients were hospitalized and examined periodically. All patients were under insulinotherapy. In twenty-six patients the disease was presented at least one year before detection. The duration of diabetes in fifty patients was from 1–3 years and in thirty-five patients from 3–5 years. Twenty-seven children were tested on the state of decompensation without ketosis, and seven children were comatose. In addition, twenty children were examined during compensation state (group 1) and twelve during decompensation with ketoacidosis (group 2) (see Table 1).

3. Results and discussion

This investigation showed that the initial disease stage (first detected IDDM) irrespective of the age is characterized by a DB_{liq} index decrease (112–200 c.u.), of more than 2 as compared with the healthy children (250±10 c.u.) [13,14,16].

Based on the DB_{liq} index, 2 groups of patients on the extensive disease stage (the duration of 1–3 and more years) were identified, although having similar clinical signs. Within the first group (fifty-eight children) the DB_{liq} index was 197±6.6 (110–260 c.u.), that is certainly lower than for healthy children ($P \leq 0.001$). In the second group (twenty-seven children) the DB_{liq} index was much higher 358±13.0 (270–493 c.u.) ($P \leq 0.001$). In children hospitalized in the comatose state, the lowest DB_{liq} index was observed 145±10 (110–180 c.u.) ($P \leq 0.001$).

The DB_{liq} index comparative analysis, for the two groups which depend on metabolic process compensation, shows that in the first group of patients more rapid metabolism compensation and clinical state improvement are observed (1/3 of children, 36%), but in the second group, metabolism process dynamics and clinical symptoms are prolonged (only 1/5 of children, 20%). It was stated, that in the first group therapeutic complex measures were highly effective. While, in the second group the insulin dose increase, for more rapid compensation, was not effective and in a number of cases lead to patient deterioration.

The DB_{liq} index in children during compensation stage and during decompensation with ketoacidosis was found to be significantly lower compared to those in healthy controls (157±12.0; 200±15.0 c.u., correspondingly, $P \leq 0.001$). Based on TLC and ozonization methods we obtained the TUD in plasma lipid fractions in children groups of different states of health. The detailed analysis of the TUD shows that the first group with the USFA deficit demonstrates a relatively normal distribution of the DB_{pow} index in the plasma lipid fractions. The total unsaturation distribution in plasma lipid fractions of

the children in group 2 shows a relative decrease of the DB_{pow} index (see Table 1) in the fraction of NEFAs (23.7, $P \leq 0.05$) with the content of their fragments in triglyceride fraction being the maximum (34.2%, $P \leq 0.05$). In children of this group the USFA–TG relation increases with the increase of the decompensation degree. This indicates lipolysis prevalence above liposynthesis.

4. Conclusions

This paper reiterates the fact that ozone reacts rapidly and completely with the ethylenic bonds of fatty acids and the cholesterol common in the blood plasma lipids. There is a parameter used by some Russian clinical biochemists also called the "double bond index". It relies on prior determination of the saturated and unsaturated fatty acids by gas–liquid chromatography (GLC) and calculations from these. The suggested calculation is carried out with the help of the compact original device "Double Bonds Analyzer" which is also extensively used for quick determination of the degree of water pollution. The important point of the DBA application to a differential diagnostic in clinical analysis is a demonstration of its sensitivity and rapidity (2–30 s). It avoids the time-consuming standard preliminary sample preparation including the extraction from silica gel, solvent removal and finally GLC analysis (2–3 h). On the other hand, within this standard technique some details of fatty acids are lost. Hence, as a diagnostic screening tool this is a promising tool at an early stage of application.

The present work shows that the DB_{liq} index depends on the compensation degree, as well as duration of the disease. The DB_{liq} index dynamic observation in children with IDDM may be used to determine the adequate treatment formulation including the insulin dosage correction, hypolipidemic and membranostabilizing medicine introduction. The DB_{liq} index may be used for the prognosis of the after-effects of treatment for all patients suffering from IDDM. The total unsaturation distributions (TUDs) in plasma lipid fractions were found to be significantly decreased when compared to those in healthy controls. In our opinion the main novelty of this paper consists of the pattern of the TUDs in plasma lipid fractions that leads to "a new biochemical criterion" of differential diagnostic in clinical analysis. It serves to indicate the metabolic disorders and decompensation in patients with IDDM. The rapidity and sensitivity are the main advantages of the suggested technique.

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