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# Evaluation of the effectiveness of complex therapy with the inclusion of a prebiotic in children with non-alcoholic fatty liver disease and excess body weight

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## Abstract

**Aim:** The present study aimed to evaluated the effectiveness of complex therapy of non-alcoholic fatty liver disease (NAFLD) with the inclusion of prebiotic lactulose on the functional state of the liver, biliary tract, intestinal microbiota, lipid and carbohydrate metabolism in 54 adolescents with excess body weight and insulin resistance (IR). **Materials and Methods:** The diagnosis of NAFLD was established based on the accepted criteria of ultrasound examination. **Result and Discussion:** Ultrasound criteria typical for liver steatosis were detected in 48% of cases, along with signs of steatosis of the pancreas and violations of the motor function of the biliary tract in 70% of patients. **Conclusion:** It has been shown that the combination of prebiotic with insulin sensitizer metformin contributes to a greater decrease in body weight, in the manifestations of steatosis of the liver and pancreas, normalization of the state of the intestinal microbiota, a decrease in IR, a level of triglycerides compared to monotherapy with metformin.

**Key words:** Children, excess body weight, insulin sensitizers, non-alcoholic fatty liver disease, prebiotics

## INTRODUCTION

According to modern data, obesity is seen as a manifestation of a complex of metabolic changes including insulin resistance (IR), arterial hypertension, atherogenic dyslipidemia, and non-alcoholic fatty liver disease (NAFLD).[1,2] According to Dedov *et al.*, every ninth adolescent is potentially at risk for developing cardiovascular diseases (CVD) and diabetes mellitus, which determines the social significance of the problem.[3]

Numerous studies have shown that one of the key organs of gastrointestinal tract involved in lipid and carbohydrate metabolism, the development of IR and dyslipidemia, is the liver.[4] Changes in the liver in obesity considered in the framework of NAFLD and can have a progressive nature, followed by the development of steatohepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma, which radically changes the prognosis of the disease.[1,5]

In addition to the liver, the microbiota of the intestine participates in the regulation of lipid metabolism both directly (due to participation in the synthesis, transformation, and destruction of endo- and exo-genous sterols) and indirectly (through changes in the enzyme hydrolysis of proteins and the synthesis of vitamins).[4] The intestinal microbiota carries out the digestion of polysaccharides with the formation of short-chain fatty acids, activates a number of enzymes that promote lipogenesis and fat deposition in adipocytes, regulate the level of gastrointestinal hormones and leptin. Changes in the composition of the intestinal microbiota contribute to the development of deeper pathological changes not only from the liver and the biliary system but also the whole organism.[6]

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There are many unresolved issues in the approaches to NAFLD treatment in children. The main methods of treatment with a proven effect remain diet and dosed physical exercise, the effectiveness of which is higher than the younger the child and the less he has an excess of body weight.[3,4,7,8] The data of retrospective observation showed low motivation and compliance of patients to long-term changes in lifestyle. In addition, it is shown that the diet compliance and dosed physical activity do not lead to the reverse development of liver fibrosis.[9]

In view of the existing recommendations for adult patients, the following areas in the treatment of NAFLD in children can be identified: (1) Decrease in IR with weight loss, performance of dosed physical activity, (2) detection and treatment of associated metabolic disorders (obesity, diabetes, and dyslipidemia), (3) application of hepatoprotective drugs with antioxidant action to protect the liver from damage, and (4) correction of the state of intestinal microflora.[10]

Prospective groups of pharmacological agents in the complex therapy of NAFLD in children include insulin sensitizers and pro- and pre-biotics. The efficacy of insulin sensitizer metformin in pediatric patients with NAFLD is shown in a large multicenter, randomized clinical trial of TONIC (2005-2010).[11] The positive effect of using pro- and pre-biotics is related to the prevention of bacterial translocation and a systemic inflammatory response caused by products of bacterial origin and endotoxinemia in NAFLD. In addition, the ability of prebiotics to stimulate differentiation of L-cells producing glucagon-like peptide in the proximal part of the colon has been demonstrated, which contributes to the reduction of IR.[6] Experimental studies in animals have shown the effectiveness of the use of prebiotics in reducing body weight and the severity of liver steatosis.[6] Thus, prebiotics, along with probiotics and insulin sensitizers, can be attributed to pathogenetically oriented therapy of NAFLD in obese children, which helps to correct the state of intestinal microbiota, metabolic disorders, and has a high safety profile. Data on the effectiveness of complex therapy in the treatment of children with excess body weight and NAFLD are absent.

The goal of the study was to evaluate the effect of complex therapy, including in addition to diet and lifestyle correction, prebiotic lactulose and insulin sensitizer metformin on the liver, biliary system, intestinal microbiota, lipid and carbohydrate metabolism in adolescents with excess body weight and NAFLD.

## MATERIALS AND METHODS

We conducted a case–control study that included 60 overweight adolescents (13.75 ± 0.56 years, body mass index [BMI] - 23.6 ± 1.09) who underwent examination on the basis of the Regional Children's Clinical Hospital (Kursk). The comparison group consisted of schoolchildren comparable in age and gender characteristics with normal BMI (18.4 ± 1.4) and 1 group of health.[12]

The detection of complaints, anthropometric and physical examination was carried out according to a standard procedure. Carbohydrate metabolism was assessed on the basis of fasting blood plasma glucose, fasting plasma insulin, C-peptide content, and calculation of IR indices (HOMA-IR index). The evaluation of lipid metabolism was carried out taking into account the European recommendations for the prevention of CVD in the clinical practice of the III revision (2003). The study of feces for dysbacteriosis was carried out in accordance with the industry standard from 2003.

The following instrumental methods of investigation were used: Ultrasonography of the abdominal cavity organs (Siemens apparatus, Sonolina SL-1) according to the conventional methods. The diagnosis of NAFLD was established on the basis of the following ultrasound criteria: The compact arrangement of echoes, increased echogenicity (the "bright liver" picture), the presence of a contrast between the hepatorenal echo sign, the depletion of the vascular pattern, and the attenuation of the ultrasonic signal.[13] The contractile function of the gall bladder (GB) was evaluated in all patients based on the determination of its volume on an empty stomach and every minute for 10 min after the appointment of the extract of artichoke leaves ("Chophytol" - 5.0 ml). Steatosis of the pancreas was diagnosed on the basis of increased size, increased echogenicity of pancreas, and the prevalence of echogenicity of pancreas over echogenicity of the kidneys.[13]

All children of the study group received recommendations for lifestyle modification and received as a basic therapy insulin sensitizer metformin ("Siofor") at a dosage of 850 mg/day for 1 month. Taking into account, the identified IR (54 children) were formed 2 subgroups (28 and 26 children, respectively). The children of the first subgroup (28 patients) to optimize the therapy were additionally assigned a lactulose preparation ("Duphalac") in a prebiotic dosage of 10.0 ml x once a day in the morning for a month. The choice of lactulose ("Duphalac") was due to a good profile of efficacy and safety in children.

The statistical analysis was carried out using the statistical package SSPS 13.0 for Windows with the definition of the arithmetic mean (M) and the standard deviation (SD). The normality of the distribution was estimated using the Shapiro–Wilk criterion. The statistical significance of the differences for data having a normal distribution was estimated using the Student's *t*-test for dependent samples, while the confidence interval for odds ratios was calculated to compare the qualitative data in the two groups. The results were evaluated as statistically significant at a probability level of $P < 0.05$.

## RESULTS

Despite the fact that patients did not have active complaints typical for liver and GB lesions, periodic pain in the upper half of the abdomen was detected in almost all children (55/91.6%). The relationship between pain and food intake was obtained (acute and fatty food provoked pain in 38/63.3% of patients) and, to a lesser extent, with psychoemotional stress. Dyspeptic syndrome was detected in 44/73.3% of patients, symptoms of intestinal dyspepsia in the form of flatulence and frequent stools (up to 2-3 times a day) were present in every third patient (17/28.3%). Among the other complaints were complaints of asthenic character, occurring in 58/96.6% of children, with a predominance of sleep disorders, characteristic of increased anxiety. Clinical examination revealed moderate pain and an increase in liver size by 1-1.5 cm from under the edge of the costal arch in more than 2/3 cases (44/73.3%). Positive GB symptoms, indicative of GB motor damage, were present in more than half of the patients (37/61.6%).

As a result of ongoing therapy in the group of children receiving the prebiotic was improved such manifestations as the need for additional efforts during defecation, a feeling of incomplete evacuation of the intestine, the stool characteristics (stool with grade 2 on the Bristol scale was revealed only in one patient, $P < 0.01$). In addition, there was a decrease in the manifestation of asthenic syndrome with an improvement in the quality of sleep, a reliable decrease in fatigue, and signs of mental exhaustion.

The parameters of body weight and BMI after the treatment in dynamics are presented in Table 1.

One month after the treatment, children showed a significant decrease in body weight, BMI, the ratio of the circumference of the waist to the circumference of the thighs, and a decrease in the thickness of the skin–fat folds. According to the results of ultrasound, there was a positive dynamics of indicators characterizing the liver, pancreas condition, and motor-evacuation function biliary tract [Table 2].

According to ultrasonography, in both subgroups, there is a significant decrease in the signs of steatosis with normalization of sizes (including I segment, [$P_1$ = 0.02, $P_2$ = 0.003]) and echogenicity (in 28.5%, $P_1$ = 0.006 and 19.3%, $P_2$ = 0.07 children, respectively) of the liver compared with the baseline indicators with unreliable differences between subgroups. Normalization of GB motor activity was noted due to improvement of its contractile function with a decrease in the number of patients with biliary sludge by 35.7% in 1 subgroup and by 23.1% in the 2nd subgroup ($P$ = 0.03). Was shown the significant positive dynamic changes in the size, ehostruktury and echogenicity of the pancreas on the background of the therapy, the maximum expressed in 1st subgroup.

When assessing the dynamics of laboratory parameters of the liver and biliary system, there was a significant decrease in the level of hepatic transferases (alanine transaminase [ALT], aspartate transaminase [AST]), total bilirubin, alkaline phosphatase (AP), gamma-glutamyltranspeptidase (GGT), compared to baseline values. In 1 subgroup, a significantly greater decrease in the level of cholestasis indicators (AP and GGT) was found, compared to the 2 subgroup (305.1 ± 96.7 IU/l vs. 383.9 ± 107.5, $P$ = 0.006 and 6.5 ± 3.9 IU/l vs. 9.7 ± 3.8, $P$ = 0.0036, respectively).

In patients with baseline AP, ALT, AST, bilirubin total, and direct fractions higher than the normative indices against the background of the therapy, the following dynamics were obtained [Figure 1].

The parameters of the lipid spectrum of blood serum in children with excess body weight after treatment are shown in Figure 2.

**Table 1:** Dynamics of body weight and BMI in children with excess body weight on the background of ongoing therapy

| Date | Comparison group, (mean±SD) | 1 subgroup (n=28) (metformin+lactulose), mean±SD | | 2 subgroup (n=26) (metformin), mean±SD | |
|---|---|---|---|---|---|
| | | Before treatment | After treatment | Before treatment | After treatment |
| Girls | | | | | |
| Weight (kg) | 48.6±6.1 | 61.7±8.7 | 56.71±5.01*/** | 61.9±8.1 | 57.2±5.9* |
| BMI, kg/m² | 20.4±1.2 | 24.48±1.16 | 21.59±1.2*/** | 24.56±1.1 | 22.32±1.3* |
| Boys | | | | | |
| Weight (kg) | 52.4±5.3 | 59.75±7.6 | 53.7±5.5*/** | 60.3±14.8 | 55.29±6.81* |
| BMI, kg/m² | 21.3±0.9 | 24.29±1.1 | 21.56±1.71* | 24.5±1.4 | 22.2±1.8* |

*Differences within subgroups before and after treatment are statistically significant ($P$<0.05), **differences between subgroups after treatment are statistically significant ($P$<0.05), SD: Standard deviation

**Table 2:** Structural and functional changes in the liver, biliary system, and pancreas on the background of therapy according to ultrasound

| Sonographic signs | Comparison group (n=30) | 1 subgroup (n=28) (metformin+lactulose) | | 2 subgroup (n=26) (metformin) | | *P* |
|---|---|---|---|---|---|---|
| | | Before treatment | After treatment | Before treatment | After treatment | |
| Size of the left lobe of the liver, mm, mean±SD | 58.1±7.2 | 61.7±11.3 | 59.86±6.7 | 62.3±11.5 | 60.4±6.8 | $P_1$=0.34<br>$P_2$=0.39<br>$P_{1,2}$=0.96 |
| Size of the right lobe of the liver, mm, mean±SD | 110.1±8.8 | 115.8±8.84 | 114.3±7.8 | 116.2±8.79 | 115.1±7.4 | $P_1$=0.62<br>$P_2$=0.65<br>$P_{1,2}$=0.54 |
| I segment, percentage of total liver volume, mean±SD | 25.4±4.2 | 29.14±4.1 | 26.65±4.9 | 29.02±4.1 | 26.35±5.1 | $P_1$=0.02<br>$P_2$=0.00<br>$P_{1,2}$=0.93 |
| Size of the head of the pancreas, sm, mean±SD | 17.9±2.4 | 19.1±3.7 | 17.6±2.4 | 19.44±3.9 | 18.5±3.2 | $P_1$=0.046<br>$P_2$=0.21<br>$P_{1,2}$=0.01 |
| Size of the body of the pancreas, sm, mean±SD | 10.8±1.7 | 12.7±3.5 | 11.1±2.3 | 12.8±3.3 | 11.9±2.7 | $P_1$=0.04<br>$P_2$=0.29<br>$P_{1,2}$=0.55 |
| Size of the tail of the pancreas, sm, mean±SD | 19.9±2.1 | 21.5±3.0 | 19.3±2.3 | 21.3±1.9 | 20.8±1.7 | $P_1$=0.03<br>$P_2$=0.32<br>$P_{1,2}$=0.01 |

$P_1$: Comparison of the results in the 1st subgroup before and after the treatment, $P_2$: Comparison of the results in the 2nd subgroup before and after the treatment, $P_{1,2}$: Comparison of the results in the 1st and 2nd subgroup after the treatment, SD: Standard deviation

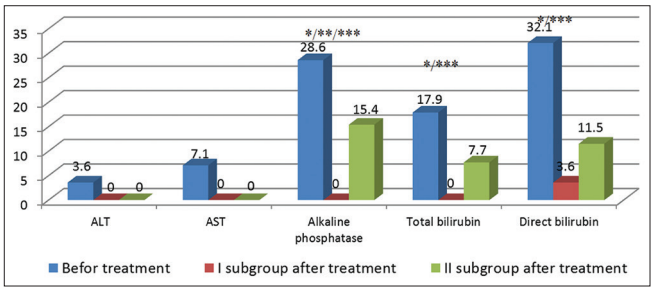


**Figure 1:** Dynamics of biochemical parameters after treatment, where the Y-axis is the number of patients as a percentage. *Comparison of results in the 1st subgroup before and after treatment, **comparison of results in the 2nd subgroup before and after treatment, ***comparison of results in the 1st and 2nd subgroup after the treatment, $P < 0.05$

When assessing the state of the lipid spectrum of the blood after treatment, a significant decrease in the initially elevated levels of total cholesterol and triglycerides (TG), low-density lipoproteins (LDLP) was detected in both subgroups, as well as an increase in the concentration of high-density lipoproteins (HDLP) in serum. When carrying out combined therapy, more pronounced positive shifts in the lipid profile with a decrease in TG, LDLP, and an increase in HDLP, a decrease in the atherogenicity index were obtained. At the same time, the indices of TG were close to those of children with normal body weight ($P = 0.08$ and $P = 0.227$, respectively).

After the treatment, a positive dynamics was obtained from the carbohydrate metabolism in both subgroups, more pronounced in the 1st subgroup with a reliable normalization of the insulin level of the blood plasma, and the C-peptide, as well as a decrease in IR values [Figure 3].

In both subgroups, after the therapy, an increase in the number of representatives of resident flora was observed, which was most pronounced in the 1st subgroup. In this case, the children who received the lactulose preparation showed a significant increase in the number of obligate flora (bifidobacteria and lactobacteria) representatives, in comparison with the second subgroup, a decrease in the amount of lactose-negative *Escherichia coli* and *E. coli* with weak enzymatic properties, a decrease in the number of conditionally pathogenic flora and fungi of the genus *Candida*.

## DISCUSSION

We showed that in adolescents with excess body weight in 78.3% of cases were noted pains in the upper part of the abdomen provoked by the intake of fatty foods. Other symptoms included flatulence, increased frequency of stools, which along with positive GB symptoms and a moderate increase in liver size, indicated involvement of the liver, and the biliary tract in the pathological process (73.3% and 61.6%, respectively). The above complaints were accompanied by the detection of ultrasound markers of liver steatosis in 48% of cases including hepatomegaly (45%), the increase in the size of 1 segment of the liver (48%), the diffuse increase in

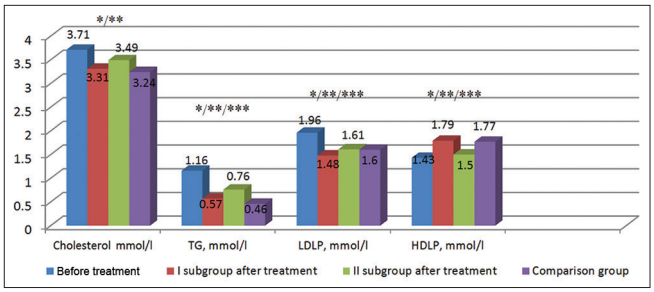


**Figure 2:** Dynamics of blood lipid profile after treatment, where triglycerides, low-density lipoproteins, high-density lipoproteins, and Y is the absolute values of the indices. *Comparison of results in the 1st subgroup before and after treatment, **comparison of results in the 2nd subgroup before and after treatment, ***comparison of results in the 1st and 2nd subgroup after the treatment, $P < 0.05$

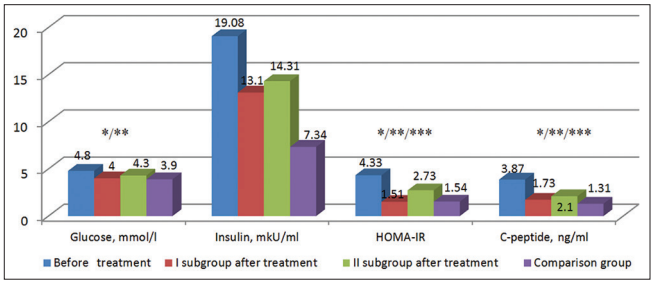


**Figure 3:** Dynamics of carbohydrate metabolism after treatment, where the Y axis - the absolute values of indicators. *Comparison of results in the 1st subgroup before and after treatment, **comparison of results in the 2nd subgroup before and after treatment, and ***comparison of results in the 1st and 2nd subgroup after the treatment, $P < 0.05$

the echogenicity of the liver (30%) and the attenuation of the ultrasound signal to the diaphragm margin (11.7%), violations of motility of the GB (70%), and a sludge syndrome in 38.3% of cases. In addition, in children with a high incidence (70%) was diagnosed steatosis of the pancreas, which indicates a common pathogenetic mechanism of ectopic fat deposition. These changes were accompanied by a complex of metabolic changes with the development of atherogenic dyslipidemia, IR, and dysbiotic disorders of the colon.

Against the background of the therapy was shown that in children receiving combination therapy body weight and BMI were significantly lower than in children who received only an insulin sensitizer ($\Delta_1$ 6.12 ± 1.08 kg and $\Delta_1$ BMI 2.73 ± 0.43 vs. $\Delta_2$ 4.88 ± 0.96 and $\Delta_2$ BMI 2.27 ± 0.25, $P < 0.05$). The decrease in body weight was accompanied by a decrease in the ultrasound signs of liver and pancreas steatosis with an improvement in the functional characteristics of the hepatobiliary system (a decrease in the level of transaminases, indicators of cholestasis - AP, GGT) to a greater extent in children receiving combination therapy with a prebiotic. Furthermore, in this group of patients, there were more pronounced positive changes in metabolic characteristics (decrease in TG, LDL level, an atherogenic index, increase in HDL level), decrease in IR index - HOMA-IR. The obtained data testify to the greater clinical and metabolic effectiveness of the combined use of the insulin sensitizer, and the prebiotic lactulose as compared to monotherapy with an insulin sensitizer, which given its good tolerability and safety, makes it attractive for use in pediatric practice.

## CONCLUSION

In children with excess body weight, despite the absence of independent complaints, 78.3% of patients has pain in the upper part of the abdomen, 73.3% - heartburn, flatulence, stool disorders, 73.3% of patients - moderate hepatomegaly, and 61.6% of them - positive GB symptoms.

The inclusion in the pathogenetic therapy of NAFLD (along with lifestyle changes) of metformin and prebiotic lactulose, in comparison with monotherapy with an insulin sensitizer, resulted in a more pronounced decrease in body weight and BMI (body mass and BMI in dynamics were Δ1 6.12 ± 1.08 kg and Δ1 BMI 2.73 ± 0.43 against Δ2 4.88 ± 0.96 and Δ2 BMI 2.27 ± 0.25, $P < 0.05$ in children of the comparison group), reduction of ultrasound signs of steatosis of the liver, phenomena of discololia, decrease in IR, normalization of lipid and carbohydrate metabolism, and improvement of the state of intestinal microbiota.

## REFERENCES

1. Bogomolov PO, Yu AH. Steatosis of the liver and non-alcoholic steatohepatitis. In: Ivashkina VT, editor. Diseases of the Liver and Biliary Tract: Monograph. Moscow: M-Vesti; 2005. p. 205.
2. Zhuravleva LV, Ogneva EV. Leptin, resistin and liver function in patients with non-alcoholic fatty liver disease and with its combination with Type 2 diabetes mellitus. Belgorod State Univ Sci Bull Med Pharm 2012;22:81-4.
3. Dedov II, Butrova SA, Savel'eva LV. Effectiveness of motivational training of obese patients. Obesityandmetabolism 2004;2:25-9.
4. Guseva AA, Gurova MM. The state of the hepatobiliary system, metabolic characteristics and quality of life in children with excess body weight and obesity. Quest Child Dietol 2013;11(2):24-8.
5. Brunt EM. Nonalcoholic steatohepatitis. Semin Liver Dis 2004;24:3-20.
6. Tehrani AB, Nezami BG, Gewirtz A, Srinivasan S. Obesity and its associated disease: A role for microbiota? Neurogastroenterol Motil 2012;24:305-11.
7. Novikova VP, Gurova MM. Modification of lifestyle-the basis of treatment of fatty hepatosis in children. Quest Child Dietol 2015;13:45-52.
8. Reinehr T, Schmidt C, Toschke AM, Andler W. Lifestyle intervention in obese children with non-alcoholic fatty liver disease: 2-year follow-up study. Arch Dis Child

2009;94:437-42.
9. Tilg H, Moschen A. Weight loss: Cornerstone in the treatment of non-alcoholic fatty liver disease. Minerva Gastroenterol Dietol 2010;56:159-67.
10. Nobili V, Alisi A, Raponi M. Pediatric non-alcoholic fatty liver disease: Preventive and therapeutic value of lifestyle intervention. World J Gastroenterol 2009;15:6017-22.
11. Alkhouri N, Feldstein AE. The TONIC trial: A step forward in treating pediatric nonalcoholic fatty liver disease. Hepatology 2012;55:1292-5.
12. Kil'diyarova RR. Nutrition for a healthy and sick child: A manual for students of medical schools. 2nd ed. Moscow: MED Press-Inform; 2015. p. 128.
13. Ou HY, Wang CY, Yang YC, Chen MF, Chang CJ. The association between nonalcoholic fatty pancreas disease and diabetes. PLoS One 2013;8:e62561.

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