Obesity also develops against a backdrop of stress, serotonin receptors of the intestinal wall [2]. Obesity and a high body mass index have been shown to be significant risk factors for the development of IBS, in addition to insufficient amount of fiber in the diet, stress, inflammation, genetic predisposition [3]. Today, IBS is one of the most common diseases of the gastrointestinal tract, and obesity is an urgent problem of endocrinology [4]. IBS according to Rome Criteria IV is defined as a chronic functional bowel disorder characterized by recurrent abdominal pain, which occurs and continues at least once a week for the last three months, associated with bowel movements, changes in frequency and consistency of the stool [5].

An important factor in improving the diagnosis of IBS is to take into account the pathogenetic factors of the disease. In recent decades, perceptions of the pathogenesis of IBS have changed significantly. Previously, IBS was considered exclusively as a psychosomatic disease, and in almost all patients it was associated with the influence of psycho-emotional factors, but today the multifactorial development of IBS is obvious. Food allergies, stress, intes-
tinal infections, hereditary predisposition, malabsorption, and disorders of bile acid metabolism are the major triggers for the development of IBS [6]. Bile acids are amphiphilic, detergent molecules synthesized by the liver that facilitate the absorption of lipids and fat soluble vitamins in the small intestine. Lithocholic and deoxycholic acids are the main bile acids present in the colon and feces. Henodexocholic and deoxycholic acids are known secretory bile acids. Increased excretion of feces and changes in the proportion of various bile acids in the feces characterize malabsorption of bile acids, which leads to diarrhea or IBS with diarrhea, which are associated with increased secretion of water and mucus in the colon, motility of the colon and membrane permeability. Bile malabsorption is known in 10–33% of patients with IBS with diarrhea or functional diarrhea [7]. However, the mechanisms of the link between metabolic regulation of bile acids and the pathogenesis of IBS remain unclear. Thus, studies that help identify specific pathogenic mechanisms for the development of IBS are relevant.

THE AIM
The aim is to investigate the effect of bile acids on the ATPase activity of the colon mucosa in patients with overweight and irritable bowel syndrome.

MATERIALS AND METHODS
All procedures with patient were performed in accordance with the informed consent of the patient “International Convention for Working with Animals” under approval of the Bioethics Committee of Danylo Halychsky Lviv National Medical University, protocol No2, 15/02, 2016.

Complex examination of 12 patients with IBS and excess body weight (mean age – 32.7 ± 1.5 years). The diagnosis of IBS was established according to Rome criteria IV [5] in the presence of recurrent abdominal pain, which was observed at least 1 day per week for the last 3 months and when there were two or more of the following symptoms: abdominal pain associated with bowel movements, pain accompanied by changing the frequency of stools or form of feces. For diagnosis inflammatory bowel pathology, CITO TEST Calprotectin-Lactoferrin (Pharmaco) was performed. We paid attention to the absence of symptoms of anxiety: fever, impurities of blood in the stool, intestinal disorders, weight loss for a short period of time, anemia, leukocytosis, acceleration of erythrocyte sedimentation rate. All patients performed measurements of height and body weight. Body mass index was calculated by the Kettle formula. According to the obtained indicators, we established the presence of excess body weight.

Isolation of subcellular postmitochondrial fraction of the patients’ colon mucous. Tissue samples were collected from patients colon during colonoscopy. Fresh samples were washed by medium A (mM): sucrose – 250, ethylene glycol tetraacetic acid (EGTA) – 1, HEPES – 10; KH2PO4 – 1; pH 7.2. Then these samples were homogenized with glassglass homogenizer at 300 rev/min for 10 min at 0-2 °C. The homogenate was centrifuged for 10 min at 3.000 g using Jouan MR 1812 centrifuge (Jouan, France) to precipitate nuclei, large cells fragments, and undestroyed cells while mitochondria remained in the supernatant 1. Next centrifugation of this supernatant 1 carried out for 10 min at 8.500 g (0–2°C). After mitochondria sedimentation, supernatant 2 was used for while ATPase activity assay. To prove a membranes presence in the post-mitochondrial fraction it was sediment for 20 min at 15.000 g.

Assay of ATPase activity. ATPase activity was determined according to the content of orthophosphate that was released after ATP hydrolysis [8,9]. At the beginning of the experiment 200 μmol post-mitochondrial subcellular fraction of patients’ colon mucous was transferred to a standard incubation medium containing (mM) NaCl – 50.0; KCl – 100.0; Tris-HCl – 20.0; MgCl2 – 3.0; CaCl2 – 0.01; pH 7.4 at 37 °C. The reaction was started by adding3 mMATP(Sigma, USA). Samples were incubated for 15 min at 37 °C with moderate shaking in a water bath. Before the end of incubation 0.4ml of medium was taken for the determination of protein content by Lowry [10]. Reaction was stopped by adding 5ml of 10% trichloroacetic acid to samples and incubating them for 30 min followed by 10 min centrifugation at 1600 g. Supernatant obtained was used to determine the content of inorganic phosphorus by the spectrophotometric method of Fiske-Subbarow [11]. We used TLC-S (Sigma, USA) at concentration 50 μmol/L for estimating their effect on ATPase activity.

Calculation of ATPase activity. The total ATPase activity of post-mitochondrial fraction of colon mucous was calculated by the difference of inorganic phosphorus in the media with different composition (supplemented with TLC-S – “experiment” or not supplemented – “control”) expressed as micromoles of inorganic phosphorus equivalent to 1 mg of protein per 1 h. Specific Na+/K+-ATPase activity was calculated as difference of inorganic phosphorus content in medium with or without ouabain (1 mM). For the determination of Ca2+/Mg2+-ATPase activity the difference between the total Ca2+/Mg2+-and Na+/K+-ATPase activity was quantified. Thapsigargin was used to calculate SERCA contribution into the total Ca2+/Mg2+-ATPase activity. Specific basal Mg2+-ATPase activity was determined in incubation medium that contained 1 mM EDTA and lack douchain. In all experiments, incubation medium was as a control for the enzymatic ATP hydrolysis.

Data analysis. The significance of differences between experimental groups was calculated using Wilcoxon-Mann-Whitney, when a data distributions were not normal. P≤0.05 was considered to be statistically significant.

RESULTS
It was found that Na+/K+-ATPase activity of subcellular fraction of colon mucous ranged from 2.32 to 15.76 and averaged (6.06 ± 1.61) μmol Pi/ mg protein per hour. TLC-S caused ranging of Na+/K+-ATPase activity from 0.74 to 13.99 and averaged (7.62 ± 1.64) μmol Pi/ mg protein per hour. Therefore, no statistically significant changes were found by
bile acid on the activity of Na+/K+-ATPase of the subcellular fraction of the colon mucous of patients with IBS. 

We observed that the Ca\(^{2+}\)-ATPase activity of EPR was ranging from 0.28 to 14.14. It was equal in average (5.88 ± 1.19) μmol Pi/mg protein per hour. TLC-S adding to the incubation medium resulted in fluctuations its activity from 0.23 to 10.89 and averaged (6.51 ± 1.20) μmol Pi/mg protein per hour. It was found that Ca\(^{2+}\)-ATPase activity of PM in control ranged from 4.84 to 15.34 and averaged (8.86 ± 1.56) μmol Pi/mg protein per hour. When TLC-S was added to the incubation medium, the activity rates of this pump ranged from 0.61 to 10.49 and averaged (6.16 ± 1.34) μmol Pi/mg protein per hour. We found that basal Mg\(^{2+}\)-ATPase activity in postmitochondrial subcellular fractions of colon mucous of the patients with IBS ranged from 0.42 to 9.24, which averaged (6.44 ± 2.02) μmol Pi/mg protein per hour. Addition of TLC-S to the incubation medium resulted in fluctuations in the activity of basal Mg\(^{2+}\)-ATPase activity in the range from 5.16 to 32.6 and averaged (23.19 ± 5.22) μmol Pi/mg protein per hour.

**DISCUSSION**

**Influence of TLC-S on Na+/K+-ATPase activity in postmitochondrial subcellular fraction of colon mucous of the patients with IBS.** As Na+/K+-ATPase plays an important role in electrolyte, water and nutrient transport across the intestinal epithelia, it is expected that the any changes in Na+/K+-ATPase activity may have a major impact in intestinal function, namely absorption and secretion. It was shown that activities of Na+/K+-ATPase was increased in children with toddler diarrhea, but Na+/K+-ATPase activity was reduced in the jeuna mucosa of patients with active celiac disease [12]. So the role of activities of Na+/K+-ATPase in IBS pathology still unknown. It is consider that perturbed bile acid metabolism plays a causal role in IBS [13]. It is possible to suppose that TLC-S might effect on activity of Na+/K+-ATPase in postmitochondrial subcellular fraction of colon mucous of the patients with IBS. But we did not found the effect of TLC-S on the activity of Na+/K+-ATPase of the subcellular fraction of the mucous membrane of the colon in patients with IBS. Our results are agreed with Hafkenscheid, who found that “the taurine derivates TC, TCDC and TDC did not influence or even enhanced the Na+/K+-ATPase activity” [14].

**Influence of TLC-S on total Ca\(^{2+}\)-ATPases activity in postmitochondrial subcellularfraction of colon mucous of the patients with IBS.** The extracellular Ca\(^{2+}\) influx is balanced by Ca\(^{2+}\) released from the cytosol by both plasma membranes and the internal Ca\(^{2+}\) -ATPases. The total Ca\(^{2+}\) -ATPases activity of the subcellular fraction consists of EPR Ca\(^{2+}\) -ATPase and plasma membrane (PM) Ca\(^{2+}\) pump EPR Ca\(^{2+}\) -ATPase play an essential role in the transport of Ca\(^{2+}\) to the EPR to replenish the calcium store, promote folding and protein maturation, lipid and steroid synthesis. It is known that TLC, as well as TLC-S, mobilizes Ca\(^{2+}\) from the intracellular pool. Thus, the main effect of TLC-S is associated with an increase in calcium cells and depletion of calcium stores. Therefore, TLC-S should affect the activity of Ca\(^{2+}\) -ATPases of the subcellular fraction of colon mucous too. But we did not observe the influence of TLC-S on Ca\(^{2+}\) -ATPase activity of the subcellular fraction of the colon mucous membrane of patients with IBS.

**Influence of TLC-S on basal Mg\(^{2+}\)-ATPase activity in postmitochondrial subcellular fractions of colon mucous of the patients with IBS.** It should to note that activity of basal Mg\(^{2+}\)-ATPase activity is coupled to H\(^+-\)translocation in PM [15,16] as well as in endosomal fraction [17]. Also in hepatocytes Mg\(^{2+}\)-ATPase activity is considered as markers of canalicularmembrane [18]. Mg\(^{2+}\)-activated ATPase of rat colon was studied in mucosa by J. Schreiner and coautors & Hafkenscheid [14, 18] and in muscle layer by Kaplia 2017 [19]. It was shown that all bile acids except cholic acid, taurocholic acid and chenodeoxycholic acid depressed the Mg\(^{2+}\)-ATPase activity in rat colon mucosa [14].

We found a statistically significant increasing of the activity of basal Mg\(^{2+}\)-ATPase activity in subcellular fraction of colon mucous under the action of TLC-S compared with the control by 3.6 times. The obtained results by the effects of TLC-S are in full agreement with the previously observed the effect of TLC-S on the activity of basal Mg\(^{2+}\)-ATPase activity in the subcellular fraction of rat liver [20]. It has been suggested that activation of basal Mg\(^{2+}\)-ATPase under the action of TLC-S may indicates to the role of the endo-lysosomal system, the so-called acid store of colon mucous of the patients in developing of pathology IBS.

**CONCLUSIONS**

TLC-S (50 μM) increased basal Mg\(^{2+}\)-ATPase in the postmitochondrial fraction of colon mucous of the patients with overweight and IBS, but had no effect on Na+-K+-ATPase and total Ca\(^{2+}\)-ATPases activity.

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Fragment of the research work: “Pathology of the respiratory, cardiovascular and digestive systems in patients with diabetes and obesity: features of pathogenesis, diagnosis and treatment”. №: IH.09.0001.16

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Conflict of interest:
The Authors declare no conflict of interest.

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Received: 17.01.2020
Accepted: 05.03.2020

A – Work concept and design, B – Data collection and analysis, C – Responsibility for statistical analysis, D – Writing the article, E – Critical review, F – Final approval of the article