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# Functions Of Apolipoprotein B (APOB) In Mechanical Jaundice And Methods Of Their Determination In Experimental Conditions

**[1]Khakulova Sh. Mukaddaskhan., [2]Tuychieva S. Dilfuza., [3]Kodirov N. Shavkat, [4]Yuldoshev A. Azamjon, [5]Kodirov Sh. Mukhammadsokhib**

[1]Khakulova Shavkatovna Mukaddaskhan - Assistant Professor, Department of Genetics and Biotechnology, Faculty of Natural Sciences, Andijan State University, 170100, Andijan city, st. University, 129, Uzbekistan.
[2]Tuychieva Sidikjonovna Dilfuza - Professor, Department of Genetics and Biotechnology, Faculty of Natural Sciences, Andijan State University, 170100, Andijan city, st. University, 129, Uzbekistan.
[3]Kodirov Nomonovich Shavkat - Head of the Department of Endosurgery, endourology, dentistry. Andijan State Medical Institute, st.Yu.Otabekov, 1, Andijon city, Uzbekistan.
[4]Yuldoshev Adkhamovich Azamjon - Head of the surgical department of the Andijan District Medical Association. Andijon city, Uzbekistan.
[5]Kodirov Shavkatovich Mukhammadsokhib – Assistant, Department of General Surgery, Andijan State Medical Institute, st.Yu.Otabekov, 1, Andijon city, Uzbekistan.

**Abstract**
Our scientific work purpose is to study the properties of lipoprotein (Apolipoprotein V) which is a protein substance of different density in the body in toxic hepatitis. This protein combines with triglycerides to form chylomicrons. In addition, they are components of enzymes, which are part of cell receptors and participate in the regulation of fat metabolism. Taking into account that these characteristics are of great importance in the diagnosis and treatment of patients, conclusions were drawn by determining ApoB in the blood 1, 3 and 8 hours after the toxic hepatitis in rats are given in this article.
**Keywords:** Apolipoprotein, chylomicron, cholesterol, triglycerides.

## 1. Introduction

Apolipoprotein B (ApoB) is a protein that forms a number of important compounds: with cholesterol - low-density lipoprotein, very low-density lipoprotein, medium-density lipoprotein and triglycerides - forms chylomicrons. It belongs to the group of apolipoproteins. They are proteins that form compounds with lipids (fat-soluble substances such as cholesterol). Such a complex of proteins and lipids performs a transport function for the movement of fat-soluble compounds in the blood and lymph water environment. In addition, apolipoproteins are components of enzymes that are part of cell receptors and participate in the regulation of fat metabolism [3].

The major form of apolipoprotein B is ApoB-48. It is responsible for the formation of chylomicrons. Chylomicrons are a complex of apolipoproteins and lipids, mainly triglycerides, which enter the body with food. ApoB-48 is located on the surface of chylomicrons, a special sphere of lipids, which ensures its stability during the passage from the intestine to the blood. Next, enzymes, lipoprotein lipase, are added to the chylomicron. They break down lipids to fatty acids, and the remnants of chylomicrons are neutralized by the liver. Thus, chylomicrons are important compounds that ensure the transfer of lipids from food to muscle, fat, and other tissues.

There are 2 forms of apolipoprotein. The first form is called apolipoprotein-B 100. This protein is synthesized in the liver and serves as a source for the formation of low-density lipoproteins (LDL) during metabolism. The second form - apolipoprotein-B 48 is synthesized by the cells of the intestinal mucosa and transports fats from the digestive tract to the liver [4]. The most common indicator for diagnosing the risk of atherosclerosis is ApoB 100, the concentration of which gives information about the amount of "bad" cholesterol in the blood.

The full function of ApoB in low-density lipoproteins has not been clearly studied. However, it is reliably known that this is the main protein without which LDL cannot be formed. In addition, it is located on the surface

of lipoproteins and acts as a ligand - an indicator for cell receptors. In other words, ApoB-48 is said to carry lipids that can be delivered to cells.

In addition to the ApoB-48 form, a form of ApoB called ApoB-100 is known. If ApoB-48 is synthesized only in the small intestine, then the liver is responsible for the formation of ApoB-100. ApoB-48 gets its name because it contains 48% of the amino acid sequence of ApoB-100.

It has been proven that high levels of low-density lipoproteins lead to the accumulation of cholesterol in the walls of blood vessels, significantly increasing the risk of developing atherosclerosis and cardiovascular diseases [2,6]. ApoB concentration in the blood is a more reliable predictor of atherosclerosis risk than total cholesterol or low-density lipoprotein cholesterol [3].

Measuring apolipoprotein levels has some advantages over measuring LDL. First, LDL is calculated mainly using the Friedewald formula, which includes several parameters. As a result, the error increases, especially for small LDL values. If the amount of LDL in the blood serum is less than 3 mmol/l, it was found that the calculation according to the Friedewald formula already has a significant uncertainty. In addition, LDL cannot be calculated in patients with triglyceride levels higher than 4.5 mmol/l. Unlike LDL, ApoB and ApoA-1 can be measured directly and accurately.

High levels of ApoB are associated with heart disease. Mutations that change the structure of Apolipoprotein B (ApoB 100 gene) cause a number of diseases: hypercholesterolemia, a hereditary (autosomal dominant) form of metabolic disorder of hypercholesterolemia. Mutations in the magnetic resonance imaging (MRI) gene are manifested by hypobetalipoproteinemia and other rare diseases [5]. Since ApoB is the main protein of low-density lipoproteins, determining its concentration determines the risk of developing coronary heart disease [1]. Along with determining the concentration of ApoB, it is important to determine the amount of apolipoprotein A1. If the ratio of ApoB concentration to apolipoprotein A1 is more than 1, the risk of developing ischemic heart disease is very high [7].

In addition to the above, it can be noted that ApoB has a systemic effect on the body. For example, it disrupts the "concentration of content" in bacteria. This condition is also called quorum sensitivity. In this process, it is a newly discovered property of bacteria to communicate and coordinate in certain ways. People with insufficient levels of ApoB have been found to be more susceptible to bacterial infections.

Based on the presence of only one apoB-100 molecule per LDL particle, it allows for successful enumeration of low-density lipoprotein particles.

Methods for measuring ApoB are currently standardized by the World Health Organization and the International Federation of Clinical Chemistry. It has been proposed to set the limits of the norm for ApoB above 1.2 g/l. For risk groups, regardless of gender, it is recommended to keep ApoB below 0.9 g/l. As for the cutoff values of the ApoB/ApoA-1 ratio, <0.9 for men and <0.8 for women can be used to determine the risk level. Normal ApoB ranges from 0.46 to 1.74 g/L for men and 0.46 to 1.42 g/L for women.

## 2. Purpose of the research

The aim of the study is to determine ApoB in the blood 1, 3 and 8 hours after ligation of the common bile duct to induce toxic hepatitis.

## 3. Materials and Methods

At all stages of the study the common bile duct was ligated 1, 3, and 8 hours after rat blood was collected. Determination of ApoB in blood plasma (determination of chromogenicity) is obtained from plasma samples prepared from Sf 60-400, Sf 20-60 and Sf 12-20 lipoproteins and LDL (d 1.019<d<1.061 kg/l).

ApoB is suspended in the sample in the presence of anti-rat ApoB antibodies. The light flux passing through the antigen-antibody complexes is directly proportional to the concentration of ApoB, which is determined by the immunoturbidimetric method [8,9].

*Composition of reagents:*

A. Reagent: Glycine buffer 100 mmol/l, sodium azide 0.95 g/l, pH 8.5.

B. Reagent: Rat anti-ApoB, sodium azide 0.95 g/l.

*Preparation of reagents.*

ApoB standard (S): Dilute with 1 ml of distilled water. It can be stable for 1 week at 2-8°C or 1 month at -20°C (by freezing once).

**Calibration curve:** ApoB standard dilutions were prepared using 9 g/L saline as diluent. To calculate the concentration at different dilutions of the standard, the concentration of the standard was multiplied by the appropriate coefficient given in the table.

| Dilution | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| ApoB standard (µl) | 10 | 20 | 40 | 60 | 80 |
| Physilogical solution (µl) | 70 | 60 | 40 | 20 | - |
| Factor | 0,125 | 0,25 | 0,5 | 0,75 | 1,0 |

Additional devices: water bath with a temperature of 37°C. 37°C thermostatic analyzer, spectrophotometer or photometer with cuvette and filter 340±20 nm.

Samples: Blood plasma obtained by standard procedure. Heparin or EDTA taken as anticoagulants. Samples cannot be frozen. Blood plasma with ApoB is stable for 7 days at a temperature of 2-8°C.

**Procedure for determining ApoB:**

1. Heat the reagents and the photometer to 37°C.
2. It is poured into the cuvette.

| | |
|---|---|
| A reagent<br>Distilled water, standard or sample | 0.8 ml<br>10 µl |

3. Mix and immediately insert the cuvette into the analyzer.
4. The resulting absorbance ($A_1$) is measured at 340 nm.

| | |
|---|---|
| B reagent | 0,2 ml |

5. Samples are placed in the cuvette analyzer. The time is determined.
6. Read the absorbance ($A_2$) at 340 nm exactly 5 minutes after the addition of reagent B.

*Calculation work. Standard curve:* Calculates the absorbance difference values of each ApoB. The distilled water (blank) value in the cuvette is used as the zero concentration standard. The concentration of ApoB in the sample is calculated by interpolating the respective absorbances ($A_2$–$A_1$) against the concentration values corresponding to the calibration curve ($A_2$–$A_1$) of the standard.

Rat ApoB (Apolipoprotein B) *ELISA Kit* was used to determine the concentration of ApoB in blood plasma.

## 4. Results and Discussion

ApoB was isolated from blood plasma, the corresponding standard curve of this reagent set was determined at a wavelength of 450 nm (Fig. 1).

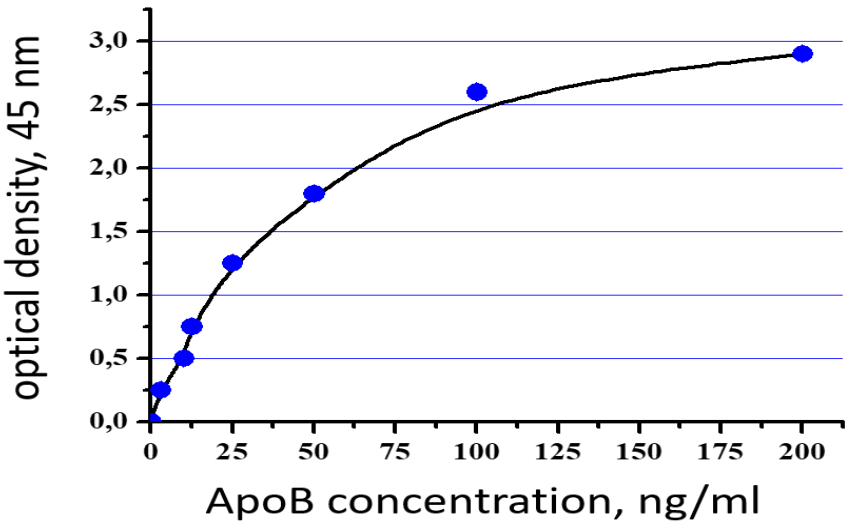


**Fig 1:** Typical standard performance curve of the ApoB (Apolipoprotein B) ELISA Kit

The normal amount of ApoB in rat blood plasma is in the range of 3,125-200 ng/ml, and the sensitivity is 1,875 ng/ml. Storage temperature (2-8°C) is important for ApoB quantification. In the conducted experiments, the amount of ApoB in rat blood plasma was determined after ligation of the common bile duct of the liver for 1, 3 and 8 hours. Ligation of the rat common bile duct for 1, 3, and 8 hours was found to cause an increase in plasma ApoB. According to the obtained results, ligation of the common bile duct for 1, 3 and 8 hours caused them to increase the amount of ApoB dramatically compared to the control (Table 1).

**Table 1:** Increase in plasma ApoB during 1, 3, and 8 hours of rat common bile duct ligation

| Experimental conditions, time (hours) | Control (ng/ml) | Experiment (ng/ml) |
|---|---|---|
| 1 | 123±12,4 | 357±17,2* |
| 3 | 127±14,2 | 567±18,0** |
| 8 | 114±15,4 | 897±28,3*** |

In the experiments, the amount of ApoB in the blood plasma was 357 ± 17.2 ng/ml after ligation of the rat hepatic bile duct for 1 hour. It caused a 2.9-fold increase in the amount of ApoB compared to the control (Fig. 2). The increase in the concentration of ApoB in the blood plasma compared to the control can be explained by the increased synthesis of ApoB by hepatocides after ligature binding.

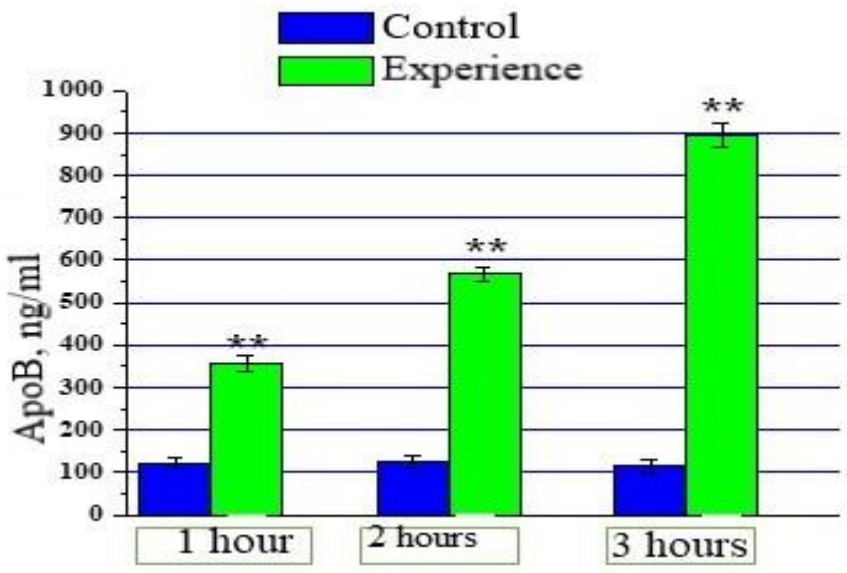


**Fig 2:** Ligation of rat hepatic common bile duct for 1, 3, and 8 hours resulted in increased plasma ApoB concentrations. (**P<0.01; n=4).

In the next part of the experiment, the rat liver was continued by ligation of the common bile duct for 3 hours. Ligation of the common bile duct for 3 hours caused an increase in the concentration of ApoB in rat blood plasma compared to control. It was found that the concentration of ApoB in the blood plasma of experimental group rats was 567±18.0 ng/ml (Fig. 2). The amount of ApoB in the blood plasma of control group rats was found to be 127±14.2 ng/ml. It was found that the amount of ApoB in the blood plasma of the rats of the experimental group increased by 4.46 times compared to the control as a result of ligation of the common bile duct for 3 hours (Fig. 2). An increase in the amount of ApoB in the plasma can be explained by an increase in the concentration of low-density lipoproteins in the blood as a result of ligation of the common bile duct.

Continuing the experiments, in the next part, the rat liver was connected with the common bile duct for 8 hours using a ligature. As a result of connecting the liver from the area close to the digestive system of the common bile duct for 8 hours, the amount of ApoB in the blood plasma was determined to be 897±28.3 ng/ml. In the rats of the control group, this indicator was 114±15.4 ng/ml. It was found that the amount of ApoB in the blood plasma of the rats of the experimental group increased by 7.86 times compared to the control as a result of ligation of the common bile duct for 8 hours (Fig. 1).

A proportional increase in the amount of ApoB in the plasma was observed when the ligature of the common bile duct of the liver was continued for a long time. It was found that this pathological process can lead to a sharp increase in the amount of lipid and protein complexes in the blood, along with the disruption of enzymatic processes in the liver. An increase in the amount of ApoB in the plasma can lead to the accumulation of cholesterol products in the walls of blood vessels of low-density lipoproteins, which can lead to a significant increase in the risk of developing atherosclerosis and cardiovascular diseases. In the future, it is an important task to create pharmacological drugs that reduce the amount of ApoB in plasma or inhibit their synthesis in the liver. It is necessary to study the mechanisms of physiological action of pharmacological preparations obtained on the basis of local plants with such hepatoprotective or cardioprotective properties and to determine their properties that correct the dysfunction of tissue cells in pathological processes.

## 5. Conclusion

1. As a result of ligation of the rat common bile duct for 1, 3 and 8 hours, an increase in the concentration of ApoB in plasma was found.
2. It was found that an increase in the amount of ApoB in plasma is associated with an increase in the amount of low-density lipoproteins.

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