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CHANGES IN THE LIPOLYTIC ACTIVITY OF PANCREATIC JUICE UNDER THE INFLUENCE OF VARIOUS PROTEINS USED AS EMULSIFIERS OF TRIBUTYRIN AND SUNFLOWER OIL

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✓ ***Resume***

In the work in vitro studied the change in the lipolytic activity of pancreatic juice under the influence of various proteins used as emulsifier's tributyrin and sunflower oil. It was concluded that the preliminary hydrolysis of proteins by pepsins in the stomach contributes not only to a further improvement of their hydrolysis under the influence of proteolytic enzymes of pancreatic juice, but also to the hydrolysis of fats under the influence of pancreatic lipase. The improvement in the lipolytic activity of pancreatic juice under the influence of various proteins depends on the length of the fatty acids of the fats.

Key words: pancreatic juice, gastric juice, proteolytic activity, protein-fat emulsion, fat hydrolysis products, proteins, tributyrin, sunflower oil.

ИЗМЕНЕНИЕ ЛИПОЛИТИЧЕСКОЙ АКТИВНОСТИ ПОДЖЕЛУДОЧНОГО СОКА ПОД ВЛИЯНИЕМ РАЗЛИЧНЫХ БЕЛКОВ ИСПОЛЬЗОВАННЫХ В КАЧЕСТВЕ ЭМУЛЬГАТОРОВ ТРИБУТИРИНА И ПОДСОЛНЕЧНОГО МАСЛА

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✓ ***Резюме***

В работе in vitro изучалось изменение липолитической активности поджелудочного сока под влиянием различных белков использованных в качестве эмульгаторов трибутирина и подсолнечного масла. Сделано заключение, что предварительный гидролиз белков пепсинами в желудке способствует не только дальнейшему улучшению гидролиза их под влиянием протеолитических ферментов поджелудочного сока, но также и гидролизу жиров под влиянием панкреатической липазы. Улучшение липолитической активности поджелудочного сока под влиянием различных белков, зависит от длины жирных кислот жиров.

Ключевые слова: поджелудочный сок, желудочный сок, протеолитическая активность, белково-жировая эмульсия, продукты гидролиза жиров, белки, трибутирин, подсолнечное масло.

ТУРЛИ ОҚСИЛЛАР ВА ЭМУЛЬГАТОР СИФАТИДАГИ ТРИБУТИРИН ВА КУНГАБОҚАР ЁҒИНИ ОШҚОЗОН ОСТИ БЕЗИ ШИРАСИ АКТИВЛИГИНИ ЎЗГАРИШИГА ТАЪСИРИ

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✓ ***Резюме***

Тадқиқотда in vitro усулида турли оқсиллар ва эмульгатор сифатидаги трибутирин ва кунгабоқар ёғини ошқозон ости бези ширасы активлигини ўзгаришига таъсири ўрганиш. Хулоса қилинганда, оқсилларни ошқозон ширасы таъсирида гидролизланганда нафақат уларни ошқозон ости ширасы протеолитик ферментлар таъсири остида гидролизини яхшилайди балки, панкреатик липаза таъсири остида ёғларни гидролизини хам яхшилайди. Турли оқсиллар таъсирида ошқозон ости бези ширасы липолитик активлигини яхшиланиши ёғ кислоталари таркибидаги занжирнинг узунлигига боғлиқ.

Калит сўзлар: ошқозон ости бези ширасы, ошқозон ширасы, протеолитик фаоллик, оқсил-ёғ эмульцияси, ёғ гидролизи махсулотлари, оқсиллар, трибутирин, кунгабоқар ёғи.

Relevance

Surfactant ingredients, such as proteins or low molecular weight emulsifiers, adsorb on the surface of the newly formed oil droplets and stabilize them. The size distribution of emulsion droplets depends on a number of factors, but one of them is how quickly protein emulsifiers are able to adsorb to the droplet surface. Fast adsorption and stabilization prevents droplet re-coalescence and results in a smaller droplet size distribution and a more stable emulsion. The structure and stability of the adsorbed protein layer is critical for the stability of the emulsion droplet [5].

The adsorption of proteins on the surface of fat droplets can reduce the activity of pancreatic lipase. The explanation for this is that proteins, due to competitive adsorption, can desorb lipase molecules from the surface of fat droplets. At the same time, the decrease in the activity of pancreatic lipase is not associated with the direct effect of the protein on the lipase enzyme, but with the competitive desorption of lipase by the protein from the substrate — the fat drop [2, 3, 6].

It was found that in protein-fat emulsions, hydrolysis of the protein layer by pepsin is the main driving force in destabilization of emulsified fat droplets. Hydrolysis of the protein layer by pepsin causes flocculation and some coalescence of fat droplets, which is most likely caused by the loss of the positive charge on the droplet surface and weakening of the adsorbed layer. Peptides that remain at the interface are unable to provide sufficient electrostatic repulsion and / or steric effects. In addition, it was also found that the action of pepsin in the hydrolysis of the adsorbed protein layer is accelerated in the presence of a salt with a high concentration [4].

Thus, with a decrease in gastric hydrolysis of proteins and their entry into the small intestine, it can help to reduce lipolysis of fats by pancreatic lipase. At the same time, an increase in the formation of protein hydrolysates by the stomach can improve the hydrolysis of fats by pancreatic lipase.

The aim of the study: to study the change in the lipolytic activity of pancreatic juice under the influence of various proteins used as emulsifiers of tributyrin and sunflower oil.

Material and methods

We used gastric and pancreatic juices obtained in chronic experiments in dogs with fasting secretion. In pancreatic juice, lipase activity was determined [1], in the presence of various proteins (casein, serum albumin, hemoglobin, gelatin, egg white, meat powder protein). As a substrate for pancreatic lipase, we used 1% tributyrin and sunflower oil emulsified with the corresponding protein in an increasing concentration from 0.1 to 1%. In order to reduce the breakdown of proteins and weaken the emmugability of the substrates used, the proteolytic activity of pancreatic juice was inhibited by using its preincubation with 0.1% soy inhibitor solution.

The study of the lipolytic activity of pancreatic juice with the studied proteins was carried out in 3 variants: 1 - without preliminary incubation with gastric juice, with 30 min of preliminary incubation with gastric juice, 3 - with 60 min of preliminary incubation with gastric juice.

Preincubation of protein substrates with gastric juice was carried out at pH 2, after which pH was neutralized to 8 with NaOH solution and addition of phosphate buffer with pH 8.2, then the substrate was incubated with pancreatic juice. In studies without incubation, distilled water was added in an equivalent volumetric amount with the spent NaOH solution and the corresponding addition of phosphate buffer.

Statistical processing was carried out by the method of variation statistics with the calculation of mean values and their mean errors, determination of the coefficient of reliability of the Student-Fisher difference (t). Differences were considered statistically significant at $p < 0.05$ and less.

Result and discussion

According to the results of the studies, it was found that the use of casein as an emulsifier, lipolytic activity is significantly reduced at a concentration of 0.1%, both when using tributyrin and sunflower oil. This decrease in activity to 0 was noted with an increase in the concentration of casein to 0.4% using tributyrin and 0.3% sunflower oil (Fig.1 A and A1).

After preliminary 30 minute incubation (preincubation) of casein of various concentrations with gastric juice and further use as emulsifiers. There was also a decrease in lipolytic activity at a concentration of 0.1%.

tributyrin and 0.4% of sunflower oil, a decrease in activity was noted to its complete absence, both when using tributyrin and sunflower oil. At the same time, the indicators were significantly higher than those without pre-incubation with gastric juice (Fig.1 A and A1).

A similar trend in the dynamics of changes in lipase activity using casein of various concentrations as an emulsifier was also observed after 60 minutes of preincubation with gastric juice. At the same time, a decrease in lipase activity, until it was completely absent, was manifested at a casein concentration of 0.6% using tributyrin and 0.5% using sunflower oil. Moreover, all indicators of lipolytic activity were significantly higher than those using tributyrin and sunflower oil without pre-incubation and higher than those indicators after 30 minutes of pre-incubation of casein with gastric juice. Also, the indicators using sunflower oil were lower than those using tributyrin (Fig.1 A and A1).

In studies using serum albumin as an emulsifier, lipolytic activity was similar to that of casein. At the same time, the decrease in activity continued, with an increase in the concentration of serum albumin until the complete absence of lipolytic activity at 0.7% tributyrin and 0.4% sunflower oil concentration (Fig.1 B and B1).

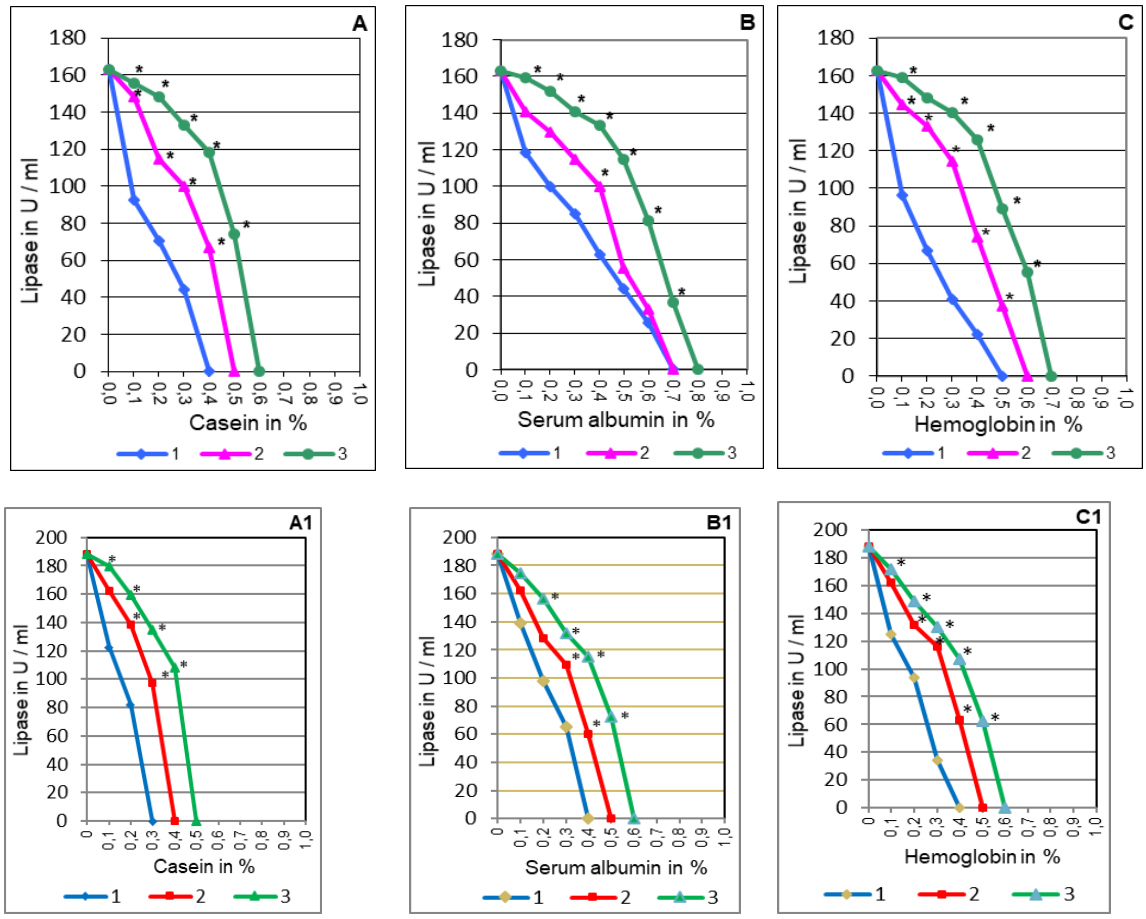

Figure 1. Influence of proteins of various concentrations after hydrolysis with gastric juice on the activity of lipase (x103) of pancreatic juice.

Note: A, B, C, - tributyrin, A1, B1, C1, - sunflower oil, 1- without preincubation with gastric juice, 2- after 30 minutes. preincubation with gastric juice, 3 - after 60 min. preincubation with gastric juice.

** - significantly different values in relation to indicators without preincubation with gastric juice.*

After preliminary 30 minute preincubation of serum albumin of various concentrations with gastric juice and their further use as emulsifiers, lipolytic activity gradually decreased to its complete absence at 0.7% using tributyrin and 0.7% using sunflower oil. These indices with the use of tributyrin and sunflower oil were significantly higher than those without preincubation with gastric juice, in addition, the indices with the use of sunflower oil were lower than those with the use of tributyrin (Fig.1 B and B1).

A similar trend in lipase activity changes using tributyrin and sunflower oil, and serum albumin of various concentrations as an emulsifier, was observed after 60 minutes of preincubation with gastric juice. At the same time, a decrease in lipase activity, until it is

completely absent, is manifested at a serum albumin concentration of 0.8% using tributyrin and 0.5% using sunflower oil. At the same time, all indicators of lipolytic activity were significantly higher than those without the preincubation of tributyrin and sunflower oil, and also higher than those indicators after 30 minutes of preincubation of serum albumin with gastric juice. At the same time, the indicators with the use of sunflower oil were lower than those with the use of tributyrin (Fig.1 B and B1).

The use of hemoglobin as an emulsifier also contributed to a significant decrease in lipolytic activity at a concentration of 0.1%, both tributyrin and sunflower oil, and this decrease continued, with an increase in concentration until the complete absence of lipolytic activity at a hemoglobin concentration of 0.5% using tributyrin and 0.4% using sunflower oil (Fig.1 C and C1).

After preliminary 30 minute preincubation of hemoglobin of various concentrations with gastric juice and further use as emulsifiers. Lipolytic activity gradually decreases with the use of tributyrin and sunflower oil, until it is completely absent at 0.6% with tributyrin and 0.6% with sunflower oil. These indicators were significantly higher than those without preincubation of hemoglobin with gastric juice using tributyrin and sunflower oil. At the same time, the indicators using sunflower oil were lower than those using tributyrin (Fig.1 C and C1).

A similar trend in the change in lipase activity with the use of hemoglobin of various concentrations as an emulsifier was also noted after 60 minutes of preincubation with gastric juice. At the same time, a decrease in lipase activity, until it was completely absent, was manifested at a hemoglobin concentration of 0.7% with the use of tributyrin and 0.5% with the use of sunflower oil. At the same time, all indicators of lipolytic activity after 60 minutes of preincubation of hemoglobin with gastric juice were significantly higher than those without preincubation of hemoglobin and higher than those indicators after 30 minutes of preincubation with gastric juice of hemoglobin, in addition, indicators using sunflower oil were lower than those using tributyrin (Fig.1 C and C1).

The use of egg white as an emulsifier contributed to a more significant decrease in lipolytic activity compared to the use of casein, serum albumin and hemoglobin. This decrease was manifested at a concentration of egg white from 0.1% and up to a complete absence of lipolytic activity at 0.3% concentration (Fig. 2 D and D1).

After a preliminary 30 minute preincubation of egg powder of various concentrations with gastric juice and further use as an emulsifier of tributyrin and sunflower oil, lipolytic activity gradually decreased at a concentration of egg white from 0.1% to its complete absence at 0.3% using tributyrin and 0 , 2% using sunflower oil.

These indicators of lipase activity using tributyrin and sunflower oil were significantly higher than those using tributyrin and sunflower oil without preincubating egg white with gastric juice. It can be seen that the indicators using sunflower oil were lower than those using tributyrine (Fig. 2 D and D1).

With the use of egg white of various concentrations as an emulsifier, after 60 minutes of preincubation with gastric juice, a similar trend in the change in lipase activity was observed. At the same the time of the decrease in lipase activity until its complete absence was manifested at an egg white concentration of 0.3% using tributyrin and sunflower oil. Moreover, all indicators of lipolytic activity were significantly higher than those without pre-incubation of egg white and the use of tributyrin and sunflower oil. Also higher than those after 30 minutes of preincubation of egg white with gastric juice. In addition, the indices with the use of sunflower oil were lower than those with the use of tributyrin (Fig. 2 D and D1).

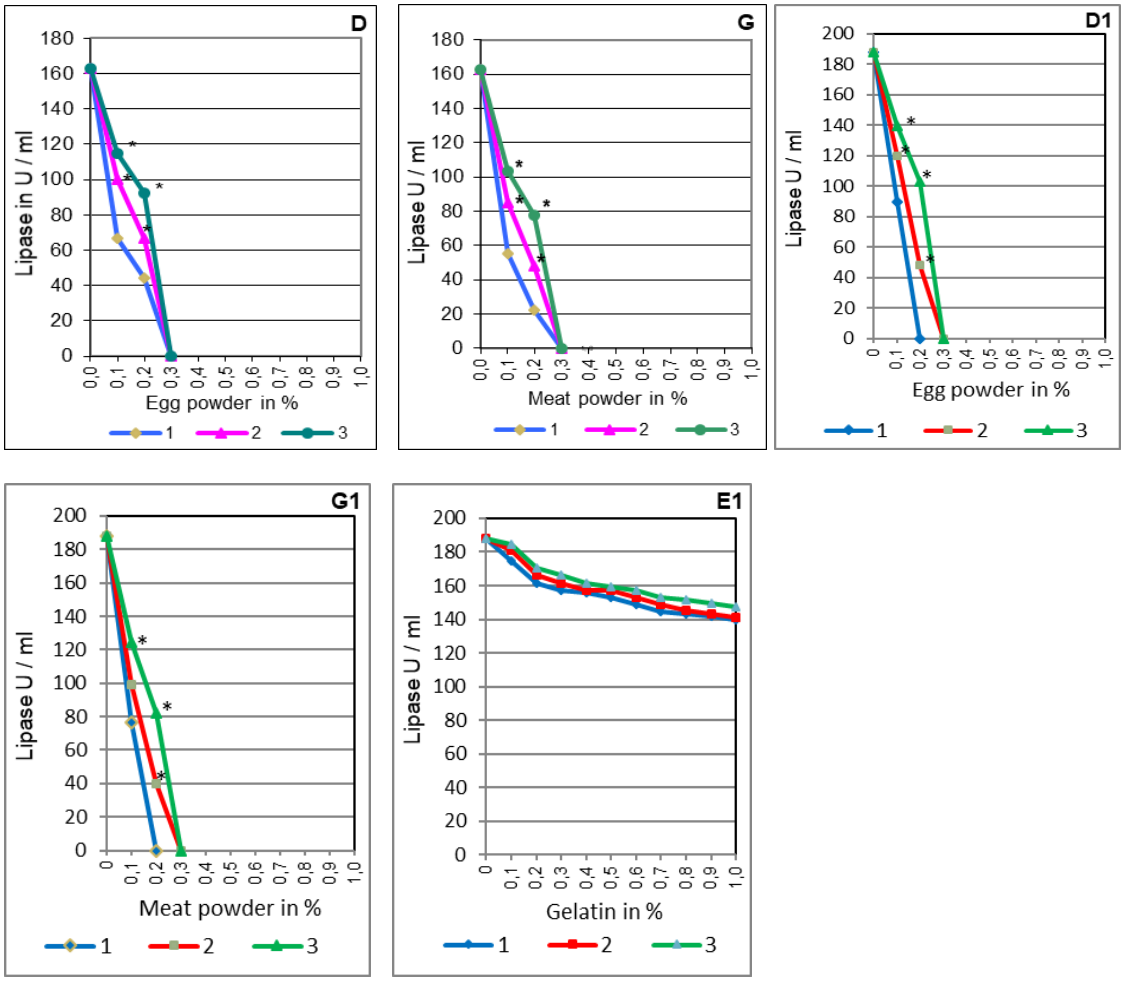

Figure 2. *Influence of proteins of various concentrations after hydrolysis with gastric juice on the activity of lipase (x103) of pancreatic juice.*

Note: D, G, E, - tributyrin, D1, G1, E1, - sunflower oil, 1- without preincubation with gastric juice, 2- after 30 minutes. preincubation with gastric juice, 3 - after 60 min. preincubation with gastric juice.

** - significantly different values in relation to indicators without prein.*

The use of meat powder as an emulsifier of tributyrin and sunflower oil also contributed to a more significant decrease in lipolytic activity compared to the use of casein, serum albumin and hemoglobin. This decrease was manifested in the same way at a concentration of meat powder from 0.1% and up to the complete absence of lipolytic activity at a concentration of 0.3% when using tributyrin and 0.2% when using sunflower oil (Fig. 2 G and G1).

After preliminary 30 minute preincubation of meat powder of various concentrations with gastric juice and further use as an emulsifier of tributyrin and sunflower oil. Lipolytic activity gradually decreased at a concentration of meat powder from 0.1% to its complete absence at 0.3% using both tributyrin and sunflower oil, which was similar to the use of egg powder, but with different dynamics. These indicators were significantly higher than those with the use of tributyrin and sunflower oil without pre-incubation of meat powder with gastric juice, and the indicators using sunflower oil were lower than those with the use of tributyrin (Fig. 2 G and G1).

With the use of meat powder of various concentrations as an emulsifier after 60 minutes of preincubation with gastric juice, a similar trend in the change in lipase activity was observed. At the same time, a decrease in lipase activity, until its complete absence, was manifested at a concentration of meat powder from 0.1% to 0.3% using tributyrin and sunflower oil. At the same time, all indicators of lipolytic activity after 60 minutes of preincubation of meat powder with gastric juice were significantly higher than those using tributyrin and sunflower oil without preincubation and higher than those indicators after 30 minutes of preincubation of meat powder with gastric juice. At the same time, the indices using sunflower oil were lower than those using tributyrin (Fig. 2 G and G1).

Lipolytic activity in studies using gelatin as an emulsifier, both tributyrtine and sunflower oil, did not significantly decrease, at various concentrations from 0.1% to 1%, both without preincubation and after 30 and 60 minutes of preincubation (Fig. 2 E and E1).

The obtained data show that all the studied proteins, except for gelatin, have an inhibitory effect on lipase in the composition of pancreatic juice, the degree of inhibitory effect for each protein is expressed differently. To the greatest extent, the inhibitory ability on lipase is expressed in proteins of egg and meat powder, in less serum albumin, as well as casein and hemoglobin. After 30 minutes and even more after 60 minutes of preincubation with gastric juice of all studied proteins, the lipolytic activity of pancreatic juice, in comparison with the indicators without preincubation, significantly increased, but not the same. The dynamics of changes in the dependence of lipolytic activity on protein concentration was different, that is, individual for each protein under study. These results indicate that gastric digestion of proteins decreases their ability to inhibit pancreatic lipase to varying degrees for different proteins. Also, the decrease in lipase inhibition by various proteins depends on the digestive ability of gastric juice. It should also be noted that the inhibitory ability of lipase proteins also depends on the physicochemical properties of fats. Fats containing short-chain fatty acids have a lower inhibitory effect on lipase proteins, and fats containing long-chain fatty acids to a greater extent. This may be due to the fact that fats containing short-chain fatty acids contribute to a lesser degree of competitive adsorption of proteins in relation to lipase on the surface of fatty droplets, compared to fats containing long-chain fatty acids.

Conclusions

Thus, the preliminary hydrolysis of proteins by pepsins in the stomach contributes not only to a further improvement of their hydrolysis under the influence of proteolytic enzymes of pancreatic juice, but also to the hydrolysis of fats under the influence of pancreatic lipase. The improvement in the lipolytic activity of pancreatic juice under the influence of various proteins depends on the length of the fatty acids of the fats.

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