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THE USE OF DIETARY SUPPLEMENTS TO REDUCE ABSORPTION OF FAT IN THE BODY

Vladimir SADOVOY, North-Caucasian Federal University, Pyatigorsk, 357502, Stavropol Territory city Pyatigorsk, st. 40 years of October; Belgorod University of Cooperation, Economics and Law, 355000, city Stavropol, st. Goleneva, 36, Russia, vsadovoy@yandex.ru *(corresponding author)*
Tatiana SHCHEDRINA, North-Caucasian Federal University, Pyatigorsk, 357502, Stavropol Territory, city Pyatigorsk, st. 40 years of October, 56, Russia, tany1812@yandex.ru
George SHCHEDRIN, North-Caucasian Federal University, Pyatigorsk, 357502, Stavropol Territory city, Pyatigorsk, st. 40 years of October, 56, Russia, reader2001@yandex.ru
Natalia LIMAREVA, North-Caucasian Federal University, Pyatigorsk, 357502, Stavropol Territory, city Pyatigorsk, st. 40 years of October, 56, Russia, nlimareva@yandex.ru

Based on the analysis of literature sources, components of food biologically active additives with preventive properties are established. With applying HyperChem (computational chemistry), we study the basic molecular features of lecithin, oleic acid and L-carnitine. Revealed that lecithin has a hydrophilic and hydrophobic properties, it is capable of forming stable emulsions and soluble on the surface section of phases. Molecule of L-carnitine has a low potential energy (48487.1 kcal / mol) and can participate in subsequent cycles of conversion of fatty acids. Formation of complexes between L-carnitine and oleic acid was investigated with the application of computational chemistry (molecular docking), the findings confirmed the possibility of formation of complex compounds, which testifies to the lipotropic effects of L-carnitine. The molecular interaction between human pancreatic lipase (receptor) and lecithin (ligand) analyzed. Based on the conducted researches, confirmed the possibility of blocking the active site of the lipase of pancreatic juice with two molecules of lecithin, this forms a stable compound with the energy of intermolecular bonds is equal to - 412.36 kkal. The expediency of using l-carnitine and lecithin for activators of the metabolism and lipolysis. Revealed that these drugs affect the absorption of fat in the intestine and prevent tumor fat. On the experimental samples (homogenized fat in water) and experimental animals used prophylactic properties of biologically active additives proven. After two hours of processing of lipid samples with pancreatic lipase and the addition of lecithin acid value of the mixture was equal to 11.91 compared with a control sample in which this indicator was equal to 77.87 mg KOH / g oil. Studies in experimental animals show, that lecithin and L-carnitine reduce obesity in experimental animals and normalize lipid, mineral, protein and carbohydrate metabolism, even in the body with increased weight.

Keywords: complex molecular compounds, L-carnitine, lecithin, lipase, molecular docking, molecular properties.

INTRODUCTION

Data from the World Health Organization indicate that more than one billion people in the world have a weight above normal. The number of people with body weight exceeding the norm by 20 % or more in developed countries is about 30 %. Obesity contributes to the development of hypertension, atherosclerosis, diabetes, cancer and others illnesses. It is, known that excess body mass by 10 % increases the average mortality rate is 30 %. 25 % of the working-age population in Russia are obese and 30 % have an increased weight (Ustinova et al.; 2004). Statistics show that obesity can considered as an important social problem that has enormous social significance. At present, special attention given to the creation of combined products with biologically active additives, providing physiological needs of the human body and building diet for the prevention of obesity.

To reduce body weight in the first place it is necessary to optimize the power mode, making it appropriate in accordance with the needs of the body. Dietary supplements are substances (or their concentrates) of natural origin or identical to those of artificial origin that do not contain drugs and intended for use with food or introduction in structure of food products (Ustinova et.al., 2008).

One of the ways of creating functional food for the prevention of obesity is the development of technologies and compositions with biologically active additives. It is important that the ingredients used raw materials, allowing to adjust the nutritive value, functional and technological properties of finished products, as well as to predict the expected therapeutic effect.

In clinical practice for the treatment and prevention of obesity, the following types of dietary supplements used: activators of lipolysis and metabolic drugs prevent tumor fat and reduce fat absorption in the intestine, having other curative properties (Sadovoy et. al.; 2017).

For the prevention of obesity is of great interest to study the mechanism of activation of the lipolysis and metabolism, effects of dietary supplementation on fat absorption in the intestine. Most commonly used for these purposes, drugs are L-carnitine and lecithin. It is also, known that these food additives have multifunctional medical and preventive properties and a positive effect on the human body.

L-carnitine is a lipotropic factors, the main use of carnitine fat loss and stabilizing healthy body weight. Dietary Supplement lecithin is able to exert an antioxidant effect, refresh the damaged cells. Lecithin brings in the cells of the drugs, vitamins, and nutrients. A lack of lecithin can cause a variety of diseases, including neurological disorders (Nikolaeva, 2002; Sas. 2006; Timoshenko and Krasilnikov, 2007). Lipotropic properties and mechanism of reducing absorption of fat in the body when used in food identified dietary supplements are not well studied.

Knowledge of the molecular properties of the spatial structure of the formed complexes fatty acids with lipotropic compounds and enzyme systems with interlocks is an important step towards the understanding of the interaction of receptors with ligands. The design of new preventive foods for individuals with excess body weight requires information about the spatial structure of the interacting activators of lipolysis and drugs inhibiting the absorption and tumor fat.

The aim of the research is to study the impact of some dietary supplements (lecithin and L-carnitine) to reduce the absorption of fats in the living body.

MATERIALS AND METHODS

Materials. Pancreatin 4300 FIP for lipase (TU Russia 49-619-79), soya lecithin (YUVIKS-Pharm, Russia), sunflower oil, refined (GOST R 52465-2005), L-carnitine (TU 9197-046-58693373-07) and other ingredients and materials that meet the requirements of the current technical documentation which are approved for use.

Methods. The degree of lipid hydrolysis was determined using the tetrametric method according to GOST R 50457-92 (ISO 660-83) [Bazarnova, 2013; Sanitary Rules and Regulations 2.2.1.3218-14; Wilson and Walker, 2015).

The experiments on laboratory animals were conducted in accordance with sanitary, sanitary-epidemiological and hygienic regulations, requirements and good laboratory practice and conformed to directive 86/609/EEC (on the protection of animals used for scientific purposes). The animals kept in a vivarium of the Stavropol State Medical University, on the standard diet in accordance with the Sanitary Rules and Regulations 2.2.1.3218-14 (GOST R 50457-92); rules of laboratory practice (USSR No. 5061-89; Rules Laboratory…), the requirements of the International Science Committee (Bulletin IKLAS, 1978) and the rules of work with the use of experimental animals (USSR Nr. 755).

Evaluation of safety and efficacy of the developed feedstuff performed on laboratory animals (white mice of both genders weighing 20 – 30 g).

For the processing of data in these studies, the following standard mathematical software packages used Statistic v.8.0, v.10.0, Statistic Neural Networks (SNN) v.4, Statistic Automatically Neural Networks Code Generator (SANN) v.8. For the modeling of quantum chemical characteristics, applied software packages HyperChem v.8 and AutoDock v.4 (Borovikov, 2001; Statistica…, 2001).

RESULTA AND DISCUSION

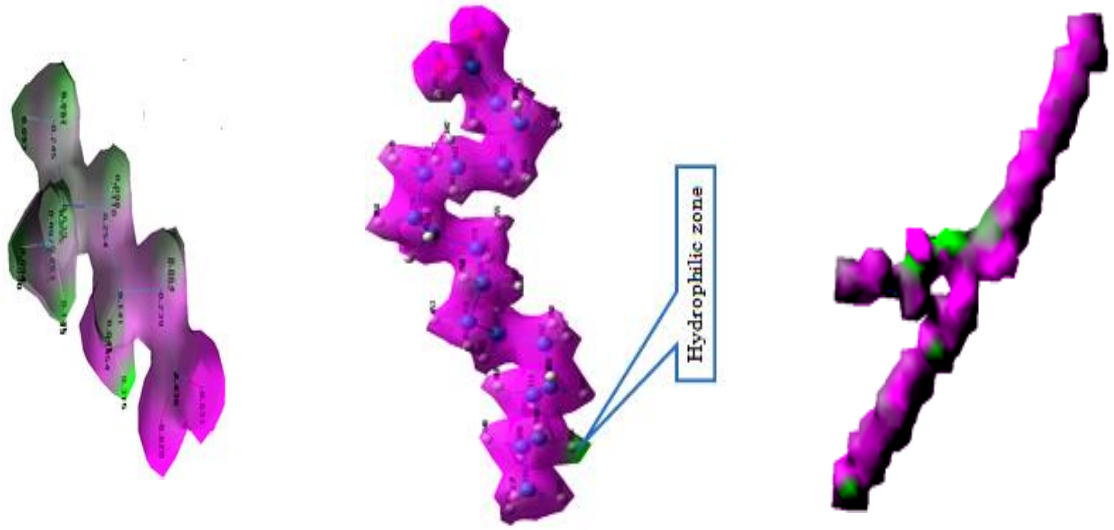
Examination of molecular properties of dietary supplements

At the initial stage to assess, the possibility of forming complex compounds have been studied the charge density distribution and molecular properties of fatty acid (oleic acid), L-carnitine and lecithin (Fig. 1). Due to the fact, that fatty acids are aliphatic monobasic compounds, which contain an unbranched chain, differ only by the number of carbon atoms, for simulation of the molecular properties used oleic acid (molecular weight 282.47 amu).

Results showed that the L-carnitine molecule (Fig. 1 a) has hydrophilic properties, as evidenced by the color scheme of the figure, the magnitude of the total charge density is equal to 0,105 eV and dipole moment – 21,53 D. The surface molecules of oleic acid hydrophobic, because the dipole moment and charge density is equal respectively equal to Debye 3.574 and the 0.06 eV, there is only one small section with hydrophilic properties (Fig. 1 b). A lecithin molecule has a low total electron density (0.01 eV) but it has hydrophobic and hydrophilic areas (Fig. 1c). The dipole moment characterizing a property of the dipole (magnitude of charge distribution on the van der Waals radius) is sufficiently small value (0 for molecules of lecithin). The results indicate that the lecithin is a surface-active agent has a high hydrophobic and hydrophilic activity that allows when used in water-fat mixtures to obtain stable emulsions at the interface of different substances.

Oleic acid has high potential energy (Total Energy equals 22.51 kcal/mol), which shows that it is an unstable compound involved in the energy process of ATP synthesis. Molecule of L-carnitine can participate in subsequent cycles

of conversion of fatty acids Potential energy shows that it is a stable compound (Total Energy – - 48487.1 kcal/mol) (Fig. 1 a). The low value of mean square gradients of the molecules indicate a correctly performed procedure the geometry optimization of the structures of all studied molecules.



a) b) c)

Figure 1. Charge distribution density in the molecules: a) L-carnitine; b) oleic acid; c) lecithin

Thus, the study of the molecular properties of biologically active additives showed that L-carnitine and lecithin are hydrophilic agents; a molecule of oleic acid with a small hydrophilic area is hydrophobic. Optimization of the spatial configuration of the structure of molecules and analysis of molecular properties necessary for studying of process of formation of the complexes by molecular docking.

Complex compound analysis

To set the level of assimilation of fat in the intestine in the presence of biologically active additives is possible by analyzing molecular docking complex compounds.

To assess the interaction of L-carnitine and oleic fatty acid (the transport and utilization of fats) and establishing a lipotropic property of biologically active additive in the application AutoDock performed molecular docking. The orientation and position of molecules relative to each other during the formation of the complex predict molecular docking. For docking of molecules in computer chemistry to model have been used the simulation. In this approach, the ligand and the receptor separated by physical distance, and the ligand in the active site of lipase proteolytic after a certain number of "steps" finds its optimal position. Steps performed to convert the studied molecules, rotation (including angular rotation) and the displacement, change of the internal structure of the ligand. At each step in the space changes, the energy state of the system simultaneously computes the molecular characteristics of each movement (Kosinsky et al., 2006; Sadovoy et. al., 2016).

In the result of molecular docking confirmed lipotropic effect of oleic acid and L-carnitine, these molecules form a stable complex, the intermolecular interaction energy is equal to – 89,08 kkal. With the use of computer simulation in chemistry revealed that L-carnitine and lipase of pancreatic juice (spatial characteristics of the lipase are taken from the PDB Bank of protein molecules) to form a sufficiently stable compound (ligand/receptor). The intermolecular energy of the complex equal – 117.91 kkal, however, does not lock the active center of lipase; the enzyme continues to play a catalytic function, which leads to the hydrolysis of lipids.

In Fig. 2 shows the results of blocking the active site (clearly visible in Fig. 2a and 2b) pancreatic lipase with lecithin before and after molecular docking.

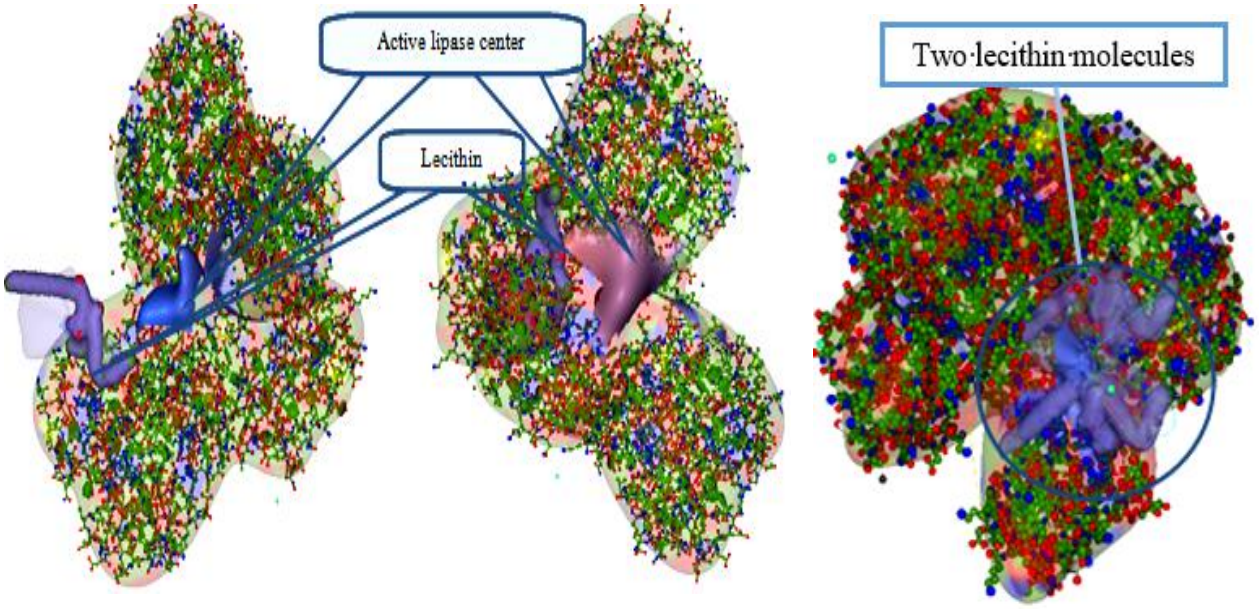
According to the obtained results of molecular docking (Fig. 2b) shows that the molecule of lecithin forms a strong complex with the enzyme, but does not complete blocking of the active site entrance or energy zone pancreatic lipase. The enzyme is able to continue to carry out the hydrolysis of lipids.

When used for simulation of two molecules of lecithin (first with one and then the other) there is a complete blocking of the active site of the enzyme. Forms a stable complex compound (the energy of intermolecular bonds is then equal to – 412.36 kcal). The active site of the lipase after docking does not exist (Fig. 2c) – the enzyme is not active.

Thus, computer modeling has shown that the use of lecithin as a food additive will allow inactivating the catalytic hydrolysis to block the active site of the enzyme, reduce absorption of fat in the gastrointestinal tract of the body.

The degree of lipids hydrolysis determined by the quantitative content of fatty acids (in the free condition, in the form of impurities). Evaluation of the hydrolytic process lead by the value of acid value. The effectiveness of biologically active additives tested on the experimental lipid compositions produced by the method of homogenization of the fat

fraction in water with the addition of products containing lecithin. The quantitative content of biologically active additives in the experimental lipid samples were determined in proportion to their physiological norm of consumption.



a) b) c)

Figure 2. A study of pancreatic lipase exposed to lecithin:
a) pancreatic lipase prior to molecular docking;
b) pancreatic lipase after docking with 1 lecithin molecule;
c) pancreatic lipase after docking with 2 lecithin molecules.

As the enzyme preparation in the experimental studies used pharmacy pancreatic lipase. The enzyme lipase is active in the range of active acidity equal to 8 – 9, adjustment of the pH of the fat-water mixture led with 0.1 N sodium hydroxide solution (Tregubova and Ismailov, 2012; Tregubova et. al., 1998). The homogeneous mixture was subjected to thermal treatment at 37ºC through each 30 minute time interval was selected samples, which determine the degree of hydrolysis of fat.

It is established that experienced the lipid sample processed with addition of lecithin after two hours of enzymatic hydrolysis at 37º C had the lowest rate of acid number equal to 11.91 mg KOH / g oil, control sample made without the use of dietary supplements had a higher degree of hydrolysis (77.87 mg KOH / g oil). Found that lecithin helps to reduce the efficiency of the hydrolytic action of pancreatic lipase. By results of researches, established that the highest activity of the drug pancreatic occurs at the initial stage of enzymatic hydrolysis (first 30 minutes).

Based on studies on the enzymatic hydrolysis of the lipid fraction of the experimental sample with a blocker of lipase, and given the consumption rates used biologically active ingredients developed the technology of obtaining and the composition of the food supplement containing lecithin. For indicators of microbiological and oxidative spoilage developed composition did not exceed the indicators established by the sanitary rules and norms № 2.3.2.1078-01.

To assess dietary and preventive properties of the developed biologically active compositions in the experimental animals was modeled obesity by increasing caloric intake with an excessive amount of lipids. The experiments the studies were conducted on white mice (weighing 20 – 30 g), divided into three groups (experience 1, experience 2 and control). Individual weight in the groups studied differed from each other by 10-15 %. Experienced groups was modeled obesity by increasing energy value of the diet (adding in food an excessive amount of lipid – 19%). The experimental group for the experiment received food enriched with fats. After two weeks in the diet of the animals of the group experience 2 were administered the researched dietary supplements. At the end of the experiment (40 days) blood of the experimental group 2 of animals on the content of lipids, cholesterol, triglycerides, protein, glucose and urea (6.58 g/l 0.80 mmol/l 0.84 mmol/l, 68.86 g/l, 6.58 mmol/l and 8.50 mg/l, respectively) differed from experimental group 1 and were close to control group.

On the basis of the conducted researches it is established that the use in the diet of specially selected dietary supplements (a certain ratio) can have on a living organism is not only preventative action, but also contribute to the treatment of various diseases. Developed in the experiment, the composition of biologically active additives lecithin and L-carnitine normalizes the metabolism of lipids, proteins, carbohydrates and mineral substances reduce the process of obesity even in the body with increased weight.

CONCLUSION

Studied the molecular properties of dietary supplements of L-carnitine and lecithin. Established that L-carnitine is a hydrophilic component; lecithin has both hydrophilic and hydrophobic properties that allows its use on the interface (oil/water). Based on the studies by the method of molecular docking revealed that two molecules of lecithin provide a lock of the active site of the enzyme pancreatic lipase, thereby forming a stable compound with the potential energy of intermolecular bonds is equal to – 412.36 kkal. Designed the Supplement with biologically active components. Studies on experimental lipid samples and experimental laboratory animals confirmed that the obtained composition normalizes metabolism in the living body and can used for prophylactic and therapeutic purposes.

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