SCHAEFFER
NUTRACEUTICALS®

Nutritional overview and health benefits research summary

October 2020

INTRODUCTION

VITAPROTEIN is based on more than 20 years of protein signal research by world-renowned American Professor **Günter Blobel**. The German-born Blobel discovered that newly formed protein molecules have internal signals that allow them to find their way to the membrane of the endoplasmic reticulum, one of the cell's inner parts, or organelles, and penetrate the membrane. This allows the proteins to get to the exact areas where they need to help cells create energy.

In the early 1970s, Dr. Blobel began describing in detail the molecular mechanisms underlying these processes. He also showed that proteins' **address label,** or **postal code,** enables them to travel to other organelles.

As it turns out, what Professor Blobel discovered is universal across all forms of life: Proteins in yeast, plants and animals behave the same way. This discovery had a major impact on cell-biology research. It showed that proteins are continuously being transported through membranes — or walls — that both separate a cell from its surroundings and separate the inner parts of a cell, the organelles.

In 1975, Blobel demonstrated that, in certain cases, amino acids in a protein act as an address label that determines where a protein will be delivered. In fact, he discovered that acid sequences determine whether a protein will be passed through the membrane out of the cell, into an organelle, or will stay in the membrane.

For his discovery that proteins have signals that govern their transport and localization in the cell, **Professor Blobel received a Nobel Prize in Physiology in 1999.**

Fascinated by Blobel's postal-code discoveries, a German scientist, **Dr. Harald Guse,** developed a patented technology based on the underlying biological concepts. It involves amino acids being encoded in a way that transforms them into a **biologically produced natural active protein carrier**.

The technology enhances the body's ability to assimilate the vitamins, minerals and natural extracts in ***VIPROACTIVE®*** diet supplements — preventive-care formulations that stimulate the body's immune and regenerative systems.

Proto Global GmbH acquired Dr. Guse's patent. Its ***SCHAEFFER NUTRACEUTICALS®*** subsidiary uses it to produce ***VITAPROTEIN®***, a biologically modified active protein carrier with encoded amino acids of natural origin. ***VITAPROTEIN®*** is the main ingredient of ***VIPROACTIVE®*** diet supplements, whose manufacturing technology is patented in Germany, with patents pending in other countries.

A renowned cardiovascular expert who believes in natural approaches to healing has been guiding ***SCHAEFFER NUTRACEUTICALS'*** research and development efforts. He is Dr. Marek Naruszewicz, a professor at the Medical University of Warsaw.

Dr. Naruszewicz has earned an international reputation for his arteriosclerosis expertise and his commitment to natural healing approaches. His insight has been invaluable to ***SCHAEFFER NUTRACEUTICALS'*** efforts to produce diet supplements of incomparable benefit and quality.

THE TECHNOLOGICAL PATENT

The patent that ***SCHAEFFER NUTRACEUTICALS®*** obtained on the use of proteins to deliver nutrients and other substances to cells has applications in food, nutraceuticals and pharmaceuticals as well as in the diet-supplement field.

An important benefit of protein delivery of nutrients is combatting abetalipoproteinemia, a disorder that interferes with the normal absorption of fat and fat-soluble vitamins from food. Protein delivery also appears to boost the body's ability to send antibodies known as gamma globulins into the blood to ensure a sound immune system.

SCHAEFFER NUTRACEUTICALS researchers tested the protein delivery vehicle both in vitro — in a lab — and in vivo — in humans. A key finding was that protein delivery led to beneficial changes in blood readings, with significant decreases in bad cholesterol and triglycerides. In addition, lab tests showed that protein delivery increased the rate at which human skeletal muscle cells multiply.

The patent covers the composition of the protein delivery system and the technology used to create it.

The first step in creating the protein that Vitaprotein uses to deliver nutrients involves adding a certain amount of bitter fennel oil to a whole chicken egg that has been liquefied. The oil's main compounds are anethole, fenchone, phellandrene and pinene. The mixing of the egg and oil is done in a specially designed machine.

Technicians use a process called electrophoresis to sort molecules in the mixture by size to ensure protein delivery-vehicle uniformity. Electrophoresis involves separating electrically charged particles by the strength of their charge. The reason this works is that larger particles carry a stronger charge.

At the same time that an electric current is applied to the egg-oil mixture, an oscillator is used to vibrate the mixture, modifying the current's frequency and amplitude. This produces the exact amino-acid sequencing that transport peptides need to deliver the protein to specific locations in cells.

During this process, the protein crystalizes, giving its molecules a permanent structure. This means they can generate the same delivery-location signals to the transport peptides consistently over time.

To ensure the egg-based granules in the final product are in the most easily digestible form possible, the protein mixture is freeze-dried at every step in the production process. This ensures an easily digestible form that can be used in food, diet supplements, nutraceuticals or pharmaceuticals.

The egg-oil mixture bolsters the protein content of foods. When it is used in a diet supplement, its task is to generate a continuous supply of the amino acids the body need to deliver nutrients to the specific locations in cells where they can be converted to energy.

The product's task as a nutraceutical is to prevent, alleviate or treat a health condition that stems from a person's inability to obtain the proper mix of vitamins and minerals from food.

As for the product's use as a pharmaceutical, two proven applications are as a treatment for **lipoproteinemia** and as a mechanism for stimulating the production of blood **gamma globulins,** which bolster the immune system.

Another potential application is as a digestive system booster. The product allows the continuous, controlled release of bitter fennel oil in the gastrointestinal tract, helping to counter harmful protease enzymes there.

THE POSTCODE DELIVERY MECHANISM

The most important finding of the Nobel Prize-winning research on which Vitaprotein products are based was that every protein has a different kind of internal signal to guide it to the cell location where it is needed to create energy.

The scientist who made this discovery, Dr. Günter Blobel, likened the internal signals to the mail codes that postal workers use to deliver letters. Because every protein has its own mail code, it can be delivered to the exact location where it is needed.

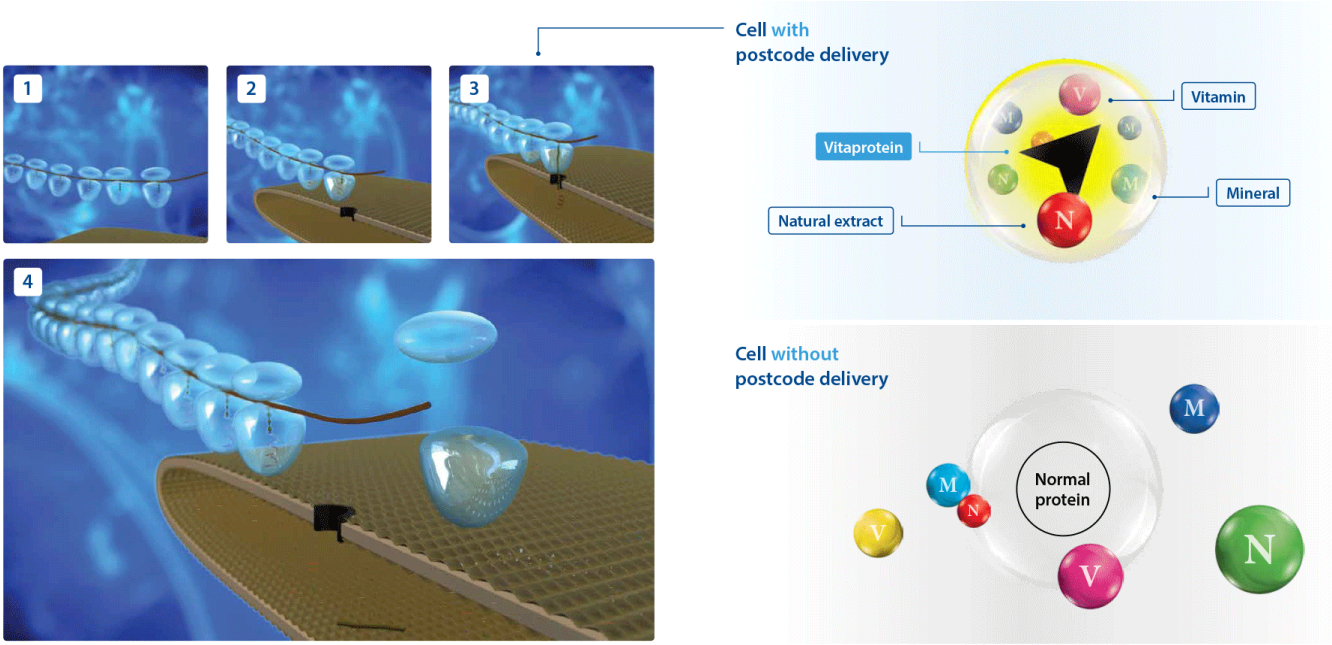
As for what constitutes a mail code, Blobel determined that in certain cases it is a protein's amino acids. In these cases, he noted, an amino acid's sequence determines if a protein will be passed through a membrane and out of a cell, into a cell component known as an organelle, or stay in the membrane.

Dr. Blobel's mail-code findings were a key reason he was awarded the 1999 Nobel Prize for Physiology, according to the Nobel selection committee.

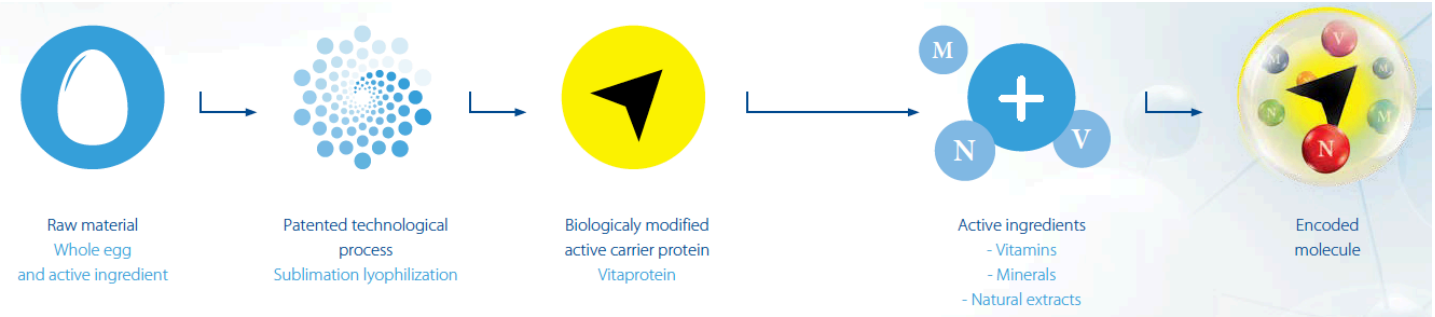
Vitaprotein products contain health-bolstering vitamins, minerals and plant extracts. They also contain signal-generating amino acids. The signals guide the product's nutrients to the precise locations in cells where they need to be converted to energy.

Ultimately the signals connect the nutrients to receptors on the membranes of the cells that need to use them. The nutrients then pass through the membranes and into the cells, where cell components called organelles metabolize them.

Because Vitaprotein products deliver nutrients to the exact locations where they need to be converted to energy, they metabolize quicker and more efficiently than other diet supplements.



RESEARCH TOWARD A PRODUCT



Research on a commercial application of what Dr. Blobel learned about protein signaling was conducted between 2000 and 2008. The main goal was creating a technology for manufacturing health-bolstering products consisting of two elements. One element was nutrients — vitamins, minerals and plant extracts. The other was a protein programmed to deliver the nutrients to the exact locations in cells where they were needed.

You can find details on both the lab and human research that led to the creation of VITAPROTEIN products below, with examples and figures. One set of findings is the results of blood tests in volunteers who took the nutrient-delivery mixture that became VITAPROTEIN. Another set was the results of blood tests among volunteers who took a placebo — non-reconstituted egg without oil.

Using the protein preparation procedure outlined in the patent, researchers mixed four tons of liquid whole-egg protein with five to 10 kilograms of bitter fennel oil.

Three hundred thirty volts of electricity were run through the mixture for periods ranging from 2 minutes, 35 seconds to 21 minutes, 15 seconds. As the current was applied, the electrodes were vibrated. The treated mixture was freeze-dried to obtain a chicken protein granulate.

Researchers also tested whole, non-reconstituted egg without bitter fennel oil to see how the mixtures compared.

The team asked a group of volunteers to consume the mixture with the fennel oil, then checked their blood readings. Eight days later they asked the same group, and a few other volunteers, to consume whole egg without the oil — then checked their blood readings.

Figures 1 and 2 show the blood test results. "0x" denotes blood-test readings before the start of the study, "x" the readings an hour after the volunteers consumed either the egg-oil mixture or egg without the oil — the placebo — and h the readings at various times beyond the first hour of consuming either the egg-oil mixture or the placebo.

An important finding was that the cholesterol of the volunteers who took the egg-oil mixture dropped an average of 6.97 percent, while that of the placebo group increased an average of 3.1 percent.

Other findings:

- Triglycerides — The triglyceride levels of the volunteers who took the egg-oil mixture fell 12 percent, while in the placebo group the levels jumped by 34 percent.

- Gamma globulins –- The egg-oil mixture led to a 38 percent increase in the level of the volunteers' immune-system-boosting gamma globulins, versus a 2.9 percent decrease in the levels of those taking the placebo.

- Insulin — Researchers tested 15 volunteers with Type 2 diabetes. Those who consumed the egg-oil mixture needed 21 percent less insulin than those who took the placebo, the team found. This indicated that the mixture countered hyperlipoproteinemia.

- Metabolism-generated heat — Overweight volunteers who took the egg-oil mixture produced 50 percent less heat from metabolism than those who took the placebo. This suggested that the metabolism efficiency of overweight people had increased to the levels of people of average weight.

- Feeling of well-being — All volunteers who took the egg-oil mixture reported a significant short-term improvement in their feeling of well-being.

Research that Professor Naruszewicz has conducted since 2008 at Warsaw Medical University support the 2000-to-2008 protein-signaling-study findings about the health benefits of an egg-oil mixture for delivering nutrients to cells. (See report on Page 9.)

One ton of liquid egg and 1.7 kilograms of copra oil were used to produce the mixture called Verum used in the Warsaw research.

01	176	37	7,7	0,44	59,6	5,5	11,7	9,7	13,5	1,47	**137**
1	176	36	8,0	0,50	58,6	5,8	11,2	9,5	14,9	1,42	**128**
10	166	33	7,9	0,50	54,7	5,1	11,6	9,4	19,2	1,21	**149**
02	115	23	7,6	10,60	54,6	7,4	12,6	11,3	14,2	1,20	**81**
2	108	24	7,6	10,60	53,8	7,4	11,9	9,9	17,0	1,16	**83**
20	113	19	8,5	10,70	50,4	7,4	12,8	9,4	20,0	1,02	**93**
03	223	39	7,5	< 0,10	62,6	6,1	10,5	10,3	10,4	1,67	**522**
3	213	44	7,4	< 0,10	61,5	6,5	10,3	10,4	11,3	1,60	**512**
30	193	46	7,2	< 0,10	56,4	7,7	9,7	10,2	16,0	1,30	**449**
04	183	36	7,1	0,14	61,7	6,2	12,5	8,3	11,3	1,61	**342**
4	195	36	7,3	0,12	60,6	6,7	11,7	8,4	12,7	1,54	**353**
40	190	39	7,5	0,16	57,3	7,9	11,0	8,6	15,3	1,34	**495**
05	173	34	7,9	0,11	61,7	7,2	9,2	8,2	13,7	1,61	**95**
5	165	35	7,9	0,16	60,5	7,9	7,6	8,4	15,6	1,53	**73**
50	156	34	7,8	0,22	58,3	8,0	8,1	7,3	18,3	1,40	**88**
06	143	25	7,5	< 0,10	56,3	6,0	9,0	9,0	19,7	1,29	**64**
6	132	22	7,0	< 0,10	54,8	6,2	8,7	8,8	21,5	1,21	**70**
60	**141**	**23**	**7,8**	**< 0,10**	**53,2**	**5,7**	**8,0**	**8,6**	**24,5**	**1,14**	**64**

Volunteer	cholesterol C	Urea	The total protein content	C-Reactive Protein (CRP)	Albumin	Alpha 1 globulin	Alpha 2 globulin	1 beta globulin	Gamma-globulin	Albumin / gamma fraction	triglycerides

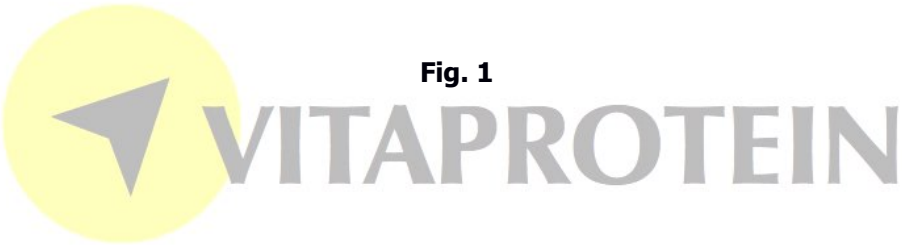


Fig. 1

Tabulated results of the "Placebo"

Proband	Cholesterin	Harnstoff	Gesamteiweiß	C-reaktives Protein	Albumin	Alpha 1 Globulin	Alpha 2 Globulin	Beta 1 Globulin	Gamma Globulin	Albumin / Gamma Fraktiom	Trglyceride
01	170	42	7,4	0,62	58,4	6,3	11,2	10,8	13,3	1,40	**72**
1	170	42	7,6	0,64	58,2	5,7	12,1	10,4	13,5	1,39	**84**
10	182	41	7,9	0,64	58,1	6,0	12,3	10,6	13,0	1,39	**88**
02	173	22	8,3	< 0,10	58,5	4,9	11,6	9,0	15,9	1,41	**75**
2	159	21	7,9	0,12	56,7	5,1	11,6	9,6	17,1	1,31	**83**
20	166	23	8,0	< 0,10	57,9	5,3	11,4	9,3	16,2	1,38	**84**
03	213	35	7,4	< 0,10	61,9	6,2	10,1	11,5	10,3	1,62	**147**
3	218	36	7,6	< 0,10	61,7	5,9	10,5	11,3	10,6	1,61	**155**
30	221	37	7,6	< 0,10	62,7	5,6	10,5	11,3	10,0	1,68	**152**
04	215	55	7,2	0,16	61,8	6,3	12,2	8,7	11,0	1,62	**227**
4	218	58	7,4	0,14	61,8	6,4	11,9	8,7	11,2	1,62	**202**
40	223	55	7,3	0,14	61,8	6,4	12,1	9,1	10,7	1,62	**209**
05	164	37	7,2	< 0,10	61,7	7,3	8,9	9,1	13,0	1,61	**44**
5	164	39	7,2	< 0,10	61,2	8,3	8,2	8,8	13,4	1,58	**69**
50	163	36	7,3	< 0,10	61,2	8,4	8,4	8,8	13,2	1,58	**86**
06	137	26	7,1	< 0,10	55,6	6,0	9,1	9,0	20,3	1,25	**96**
6	135	23	7,0	< 0,10	55,0	6,2	9,1	9,4	20,3	1,22	**76**
60	137	23	7,1	< 0,10	55,7	6,2	8,9	9,7	19,5	1,26	**80**
07	139	35	7,6	0,14	63,0	5,9	8,2	9,1	13,9	1,70	**154**
7	136	34	7,4	0,13	62,4	5,9	7,6	9,7	14,3	1,66	**116**
70	**142**	**35**	**7,6**	**0,10**	**62,5**	**5,4**	**8,1**	**10,4**	**13,6**	**1,67**	**122**

Volunteer	*cholesterol C*	*Urea*	*The total protein content*	*C-Reactive Protein (CRP)*	*Albumin*	*Alpha 1 globulin*	*Alpha 2 globulin*	*1 beta globulin*	*Gamma-globulin*	*Albumin / gamma fraction*	*triglycerides*

Fig. 2

AMINO ACID COMPOSITION

Amino Acid	Content per 100 g VITAPROTEIN
Alanine	2.69 mg
Arginine	2.88 mg
Aspartic acid	4.76 mg
Cysteine	1.09 mg
glutamic acid	6.26 mg
Glycine	1.56 mg
Histidine *	1.09 mg
Isoleucine *	2.54 mg
Leucine *	3.97 mg
Lysine *	3.43 mg
Methionine *	1.51 mg
Phenylalanine *	2.48 mg
Proline	1.78 mg
Serine	3.48 mg
Threonine *	2.29 mg
Tryptophan *	0.59 mg
Tyrosine	1.93 mg
Valine *	2.87 mg

(*) essential or indispensable amino acid that human organism is unable to synthesize and must be supplied in the diet.

NUTRIENTS IN 100 g VITAPROTEIN

Calorific value (kcal)	580 kcal = 2.426 kJ
protein	54 g
Carbohydrates	4.9 g
Fat	38 g

SCIENTIFIC RESEARCH

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Report

1. Material.

Subject of the test was Vitaprotein in form of lyophilized powder.

2. Preparing of the tested substance

Tested preparation was dissolved in phosphate-buffered saline (PBS) and next in the culture medium to obtain solutions at a concentration 5; 1; 0,5; 0,25 i 0,125 mg/mL. Such prepared solutions were added on cell culture plates. The same volume of PBS was added to control cells.

3. Experimental materials

Cell culture medium SkGM-2 – CC-3245 (Lonza); Neutral Red – N4638 (Sigma-Aldrich); Thiazolyl blue tetrazolium bromide (MTT) – ab146345 (Abcam); BrdU Cell Proliferation ELISA Kit – ab126556 (Abcam); Human SIRT1 ELISA Kit – ab171573 (Abcam), Casein from bovine milk – C3400 (Sigma-Aldrich).

4. Cell line

In the study human skeletal muscle (SkMC) cell line (CC-2561) purchased from Lonza was used. Cells were cultured in the SkGM medium in 37°C, 95% humidity, 5% CO2. For each experiment cells were seeded on 96-well culture plates at a density 1x103 per well.

5. Study design

The cells were incubated in the presence of tested preparation for 24, 48 and 72 hours. Then, proliferative activity using MTT test and BrdU incorporation test as well as cytotoxicity using neutral red uptake (NRU) test were determined. Intracellular Sirt1 level was determined using ELISA test. All tests were

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performed in three independent experiments. In addition, the cells were incubated in the presence of casein from bovine milk at the same concentration, for comparative purposes.

a. MTT test

SkMC cells were incubated in a presence of tested preparation for 24, 48 and 72 hours. Then, culture medium was removed, MTT solution was added and cells were incubated for another 3 hours. Next, MTT solution was removed, cells were washed with PBS, a formed crystals were dissolved in 0,04M HCl in isopropanol. Absorbance was measured on microplate reader Biotek Synergy4 at λ=570 nm.

b. NRU test

SkMC cells were incubated in a presence of tested preparation for 24, 48 and 72 hours. Then, culture medium was removed, NR solution was added and cells were incubated for another 3 hours. Next, NR solution was removed, cells were washed with PBS, and formed crystals were dissolved with NRU desorb (water-ethanol-acetic acid 50:49:1). Absorbance was measured on microplate reader Biotek Synergy4 at λ=540 nm.

c. BrdU incorporation

SkMC cells were incubated in a presence of tested preparation for 24, 48 and 72 hours. Then, BrdU solution was added and cells were incubated for another 2 hours. Detection of BrdU incorporation was performer using ELISA test according to manufacturer protocol. Absorbance was measured on microplate reader Biotek Synergy4 at λ=450 nm.

d. Intracellular Sirt1 level

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SkMC cells were incubated in a presence of tested preparation for 24, 48 and 72 hours. Then, cells were lysed and intracellular Sirt1 level was assessed using ELISA test according to manufacturer protocol. Absorbance was measured on microplate reader Biotek Synergy4 at λ=450 nm.

e. Results preparation

Results were presented as mean from three experiments with standard deviation (SD) in comparison to control cells.

6. Results

Tested preparation did not exhibit cytotoxic effect on human skeletal muscle cells in vitro at indicated concentrations. Beneficial effect of tested preparation on proliferative activity of the cells was observed.

Incubation of the cells in presence of tested preparation caused increase of cell proliferation. Observed effect was concentration-dependent as well as time-dependent, reaching maximum at 72 hours [Figure 1]. In MTT test, after 72 hours of incubation increase of cells proliferation by 6,15%, 13,74% i 23,25% in comparison to control cells was observed for concentration of 0,25 mg/mL, 0,5 mg/mL and 1 mg/mL, respectively [Figures 2a, b, c]. In NRU test, after 72 hours of incubation increase of cells number by 5,40%, 13,17% i 25,05% in comparison to control cells was observed for concentration of 0,25 mg/mL, 0,5 mg/mL and 1 mg/mL, respectively [Figures 3a, b, c]. In BrdU test, after 72 hours of incubation increase of BrdU incorporation by 26,77%, 42,06% and 65,73% in comparison to control cells was observed for concentration of 0,25 mg/mL, 0,5 mg/mL and 1 mg/mL, respectively [Figure 4].

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Incubation of the cells in presence of tested preparation did not cause statistically significant increase of intracellular Sirt1 level after 24, 48 and 72 hours [Figures 5a, b, c].

Additionally, incubation of tested cells with casein at the same concentration caused lower increase of proliferation [Figures 6a, b, c] and cells number [Figures 7a, b, c] than incubation with tested preparation.

7. Conclusions

Performed study demonstrated beneficial effect of the preparation on the human skeletal muscle cells in concentration-dependent manner, however, without significant impact on intracellular Sirt1 level. Observed effect was significantly stronger than of casein from bovine milk at the same concentration, which was used for comparative purposes.

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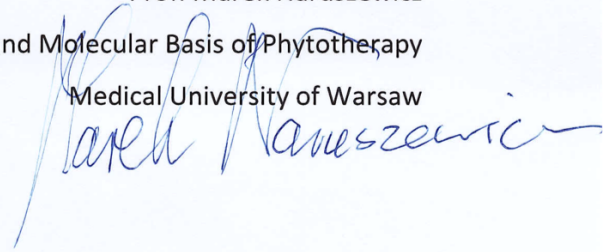

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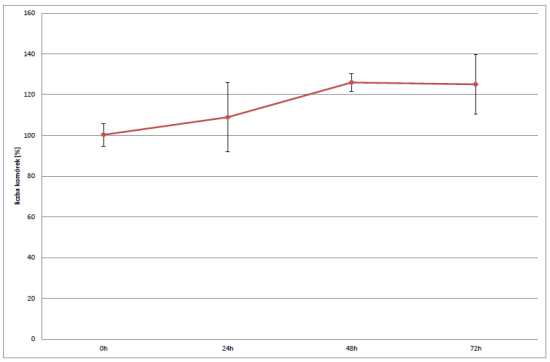
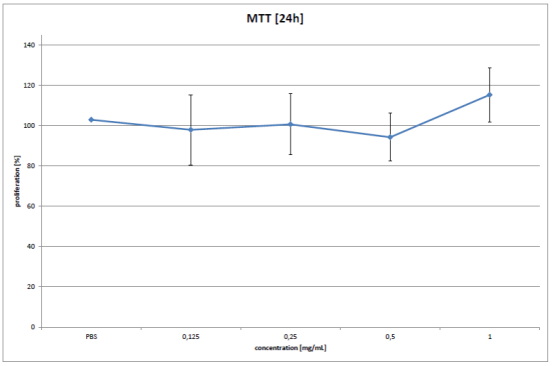
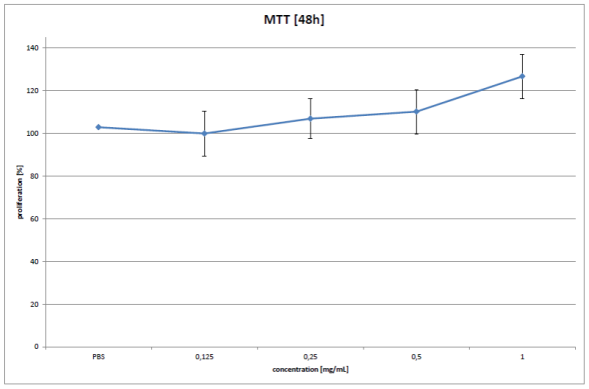
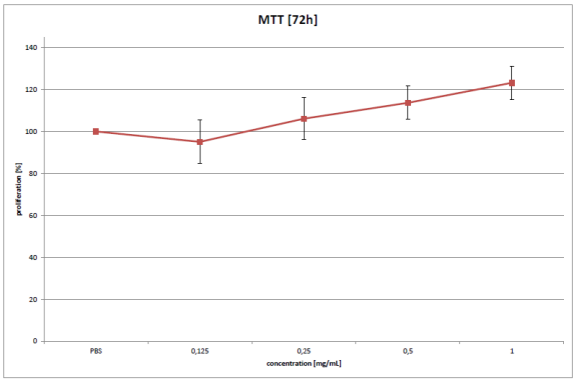
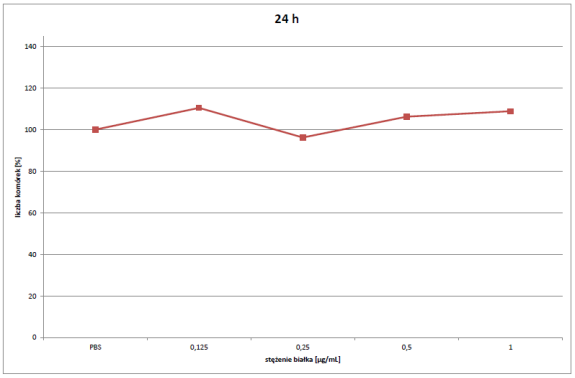
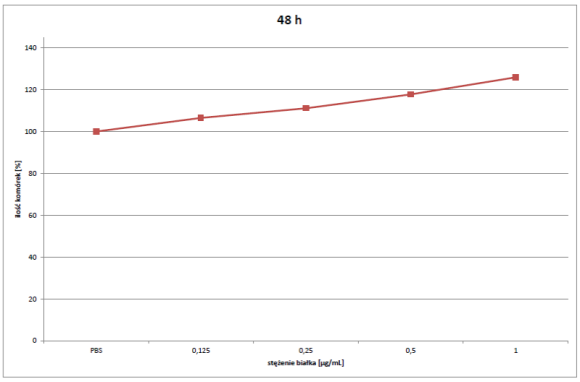


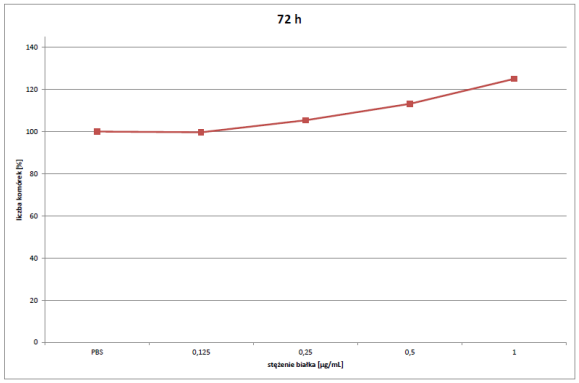


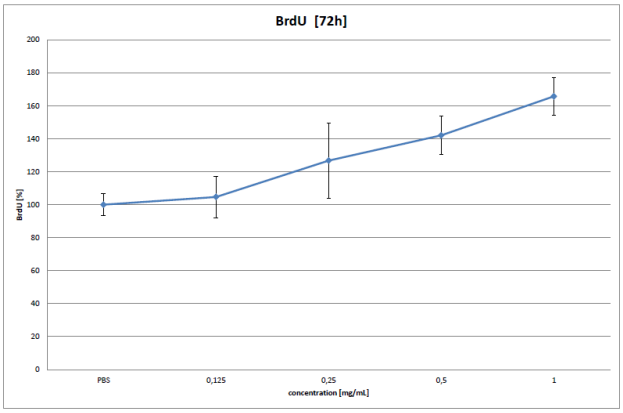


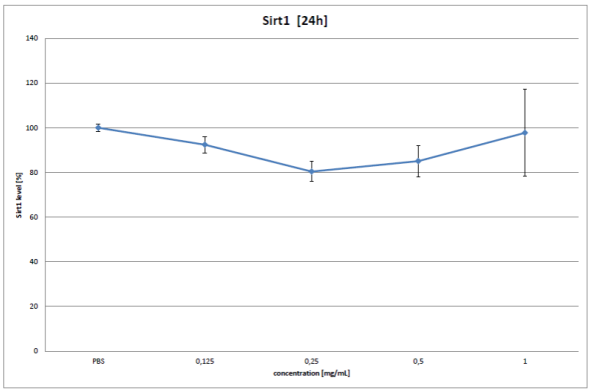


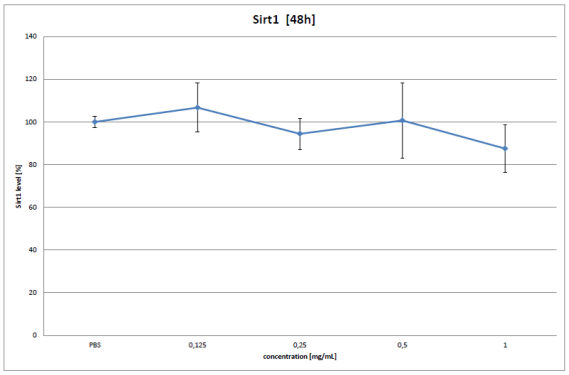


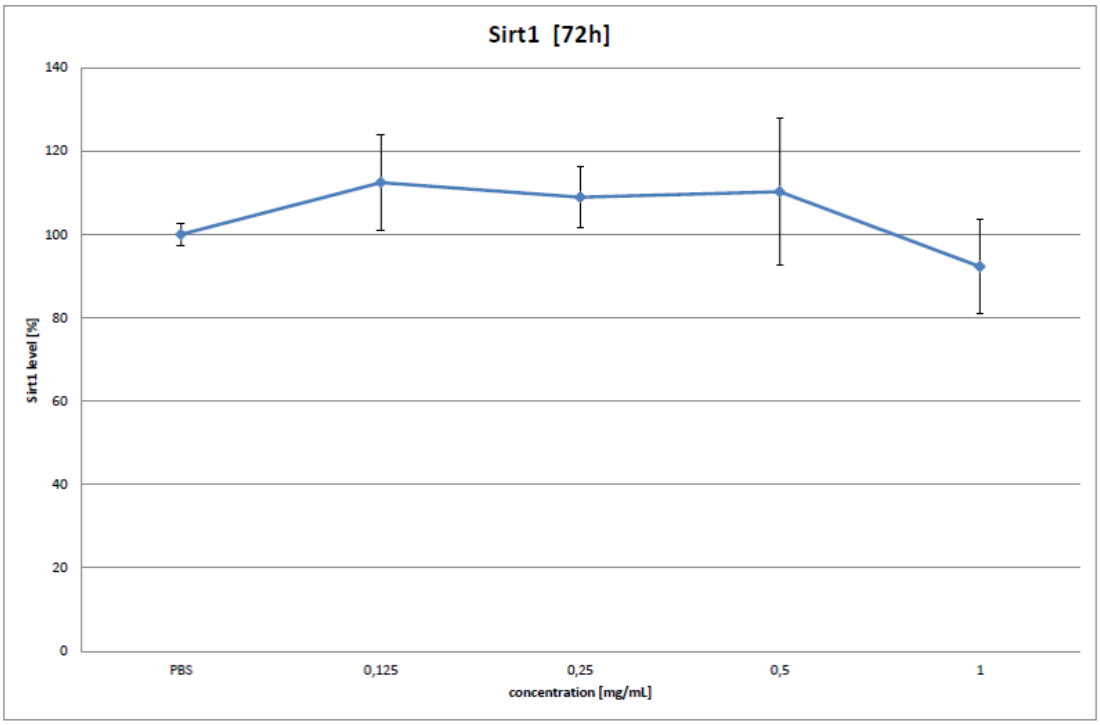


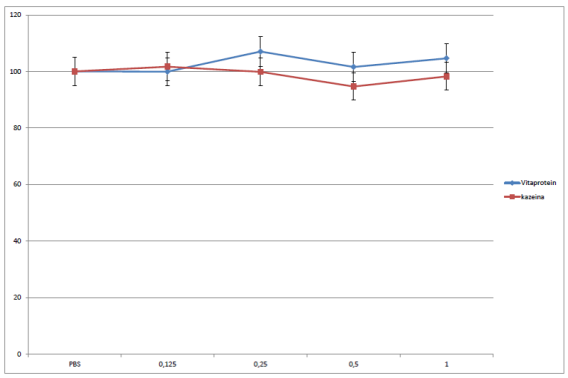


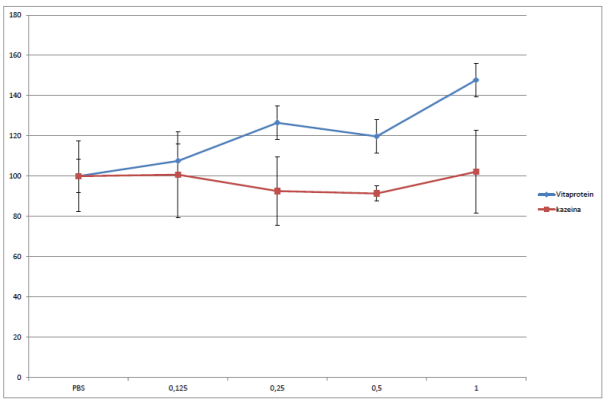


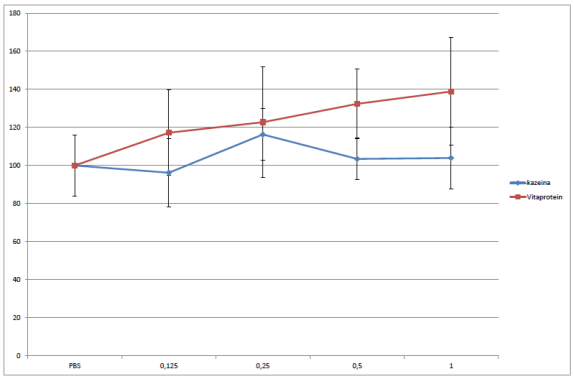

Sirt1 [24h]
Sirt1 level [%]
140
120
100
80
60
40
20
0
PBS
0,125
0,25
0,5
1
concentration [mg/mL]

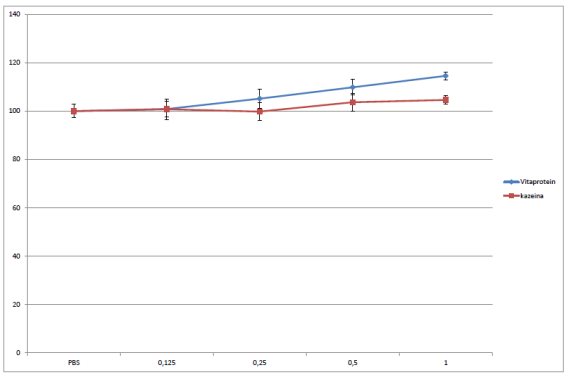

Sirt1 [48h]
Sirt1 level [%]
140
120
100
80
60
40
20
0
PBS
0,125
0,25
0,5
1
concentration [mg/mL]

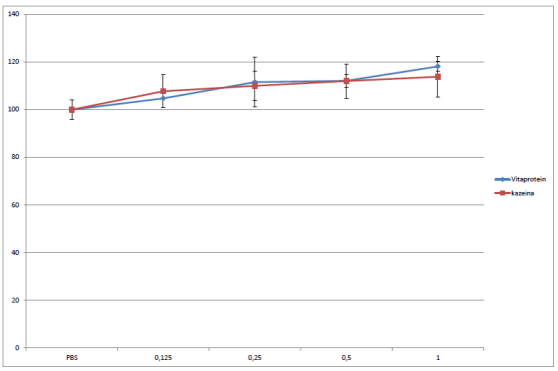

Sirt1 [72h]
Sirt1 level [%]
140
120
100
80
60
40
20
0
PBS
0,125
0,25
0,5
1
concentration [mg/mL]

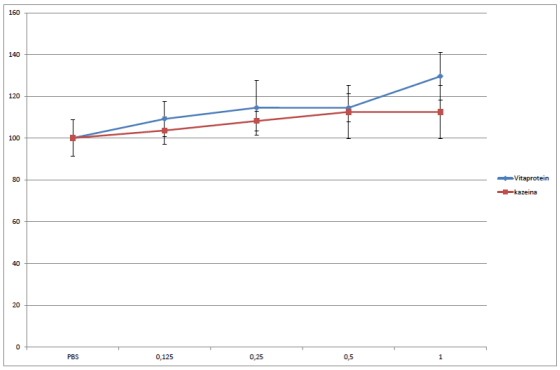

120
100
80
60
40
20
0
PBS
0,125
0,25
0,5
1
Vitaprotein
kazeina


180
160
140
120
100
80
60
40
20
0
PBS
0,125
0,25
0,5
1
Vitaprotein
kazeina


180
160
140
120
100
80
60
40
20
0
PBS
0,125
0,25
0,5
1
kazeina
Vitaprotein


140
120
100
80
60
40
20
0
PBS
0,125
0,25
0,5
1
Vitaprotein
kazeina


140
120
100
80
60
40
20
0
PBS
0,125
0,25
0,5
1
Vitaprotein
kazeina


160
140
120
100
80
60
40
20
0
PBS
0,125
0,25
0,5
1
Vitaprotein
kazeina