

IDEAL PHARMA PEPTIDE

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—
RELEVANT RESEARCH

**BCAA IPH AEN
AMINO ACID
PEPTIDE COMPLEX**

2018

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Ideal Pharma Peptide

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Ideal Pharma Peptide GmbH is an innovative European company which has developed a number of unique IPH peptide complexes for food, sports and beauty industries. This development is based on the international scientific experience, knowledge concerning peptides and innovative production technologies.

Along with the above the company develops and improves analysis methods of peptide complexes, it conducts research on their efficacy in their pure form as well as in derived products.

Production of peptides and based on peptide complexes is a sophisticated, high-tech process which implies presence of complex biotechnological innovations as well as serious scientific and laboratory facilities. Not all companies possess such impressive resources but this problem can be resolved by investments.

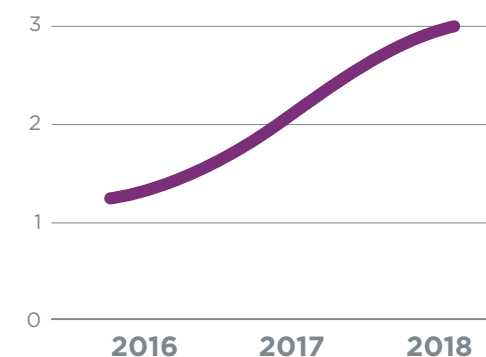
Investing in innovative products is the best decision to obtain economic growth and maximize profit of your company. Standard products presuppose high competition and therefore low added value for the manufacturing company.

We offer our partners an opportunity to increase rentability, improve technology and make the most of the production capacity via lower expenses. There is no need to purchase raw material, address the issue of stocking material with different shelf lives, acquire additional components, etc.

By receiving ready-made innovative complexes, our partners obtain a business solution with a high added value of the final product.

In today's fast-paced world, investments in innovation contribute to the company's competitiveness and lead to seizing a significant market share. The first companies entering the market with the help of innovative products get competitive advantage and maximize their profits.

The amount of global market of products containing peptides, bln \$



Peptide History

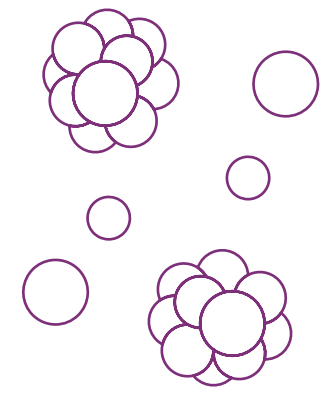
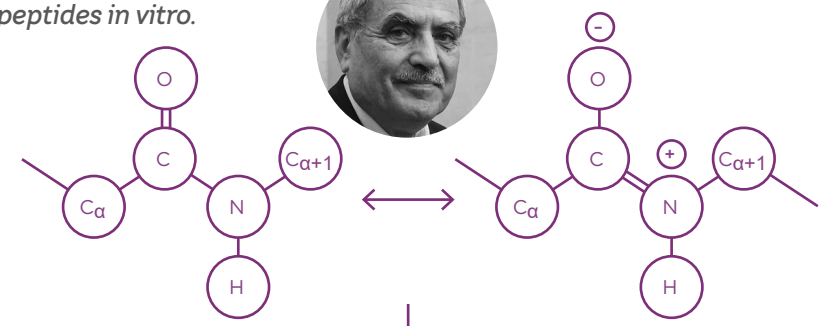
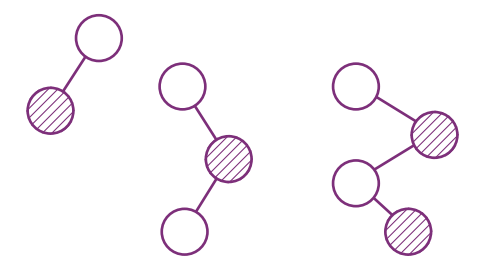
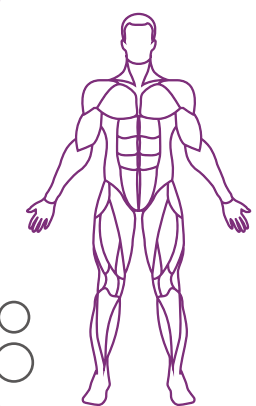
The term "peptide" was introduced by the German organic chemist **Hermann Emil Fischer** at the beginning of the 20th century.

The scientist was the first to discover this substance and to put forward a hypothesis that peptides consist of chains of amino acids formed by certain bonds. Already in 1902 he obtained irrefutable proof of existence of peptide bonds, in 1905 he invented a method of peptide synthesis in vitro. As a result scientists began to conduct detailed studies of the structure of various substances, develop methods of breaking down polymeric compounds into monomers and create peptides in vitro.

Professor **Jean Martinez** being a talented scientist in the field of Medical Chemistry Pharmacology and Peptide Biology provided valuable insights in the field of Concept of Organic and Peptide Syntheses as well as of the development and synthesis of various selective neuropeptide prototypes and biomaterials containing biomolecules with strong effects.

Since the end of the last century, peptides have been widely used in sports, replacing hormones of the 90s of the 20th century. For an athlete to gain strength, endurance and rapid recovery, training and proper nutrition is not sufficient. He/she definitely needed pharmacological agents providing a selective effect on a range of hormones, yet not violating the general hormonal background.

Science is going from discoveries to practice in leaps and bounds: just over a hundred years has passed since the discovery of peptides, and they are already inherent in our lives.



For the first time...

Once upon a time...

Recently...

Today...

Tomorrow...

During the 70s of the 20th century another important event took place: Paul Niehans' students managed to "conserve" embryonic cells for the first time in history.

It is also known that Emil Fischer's student Dr. **Otto Warburg**, biochemist and a Nobel laureate in the field of Biology proved through an experimental method that cell cultures which underwent such treatment retained their main properties. After those discoveries the world's first cellular cosmetics appeared which later grew into an extensive and promising area of dermal reductants.

The Russian scientist, holder of Habilitation Degree in Medicine Professor **Vladimir Khavinson** conducted researches in Biochemistry, Gerontology and Immunology which allowed him to develop efficacious technologies against ageing as well as to correct the functioning of all the body systems including the technology of peptide bioregulation. Professor Khavinson's innovative elaboration in the field of synthesis research and short peptide implementation has been accepted as revolutionary.

The essential difference of peptide complexes developed by **Ideal Pharma Peptide GmbH** is the safety and simplicity of the peptide complexes application, they are composed of innovative ingredients and are embedded in the usual products for sports (BCAAs, Arginine, Glutamine, Carnitine, Creatine, Taurine, etc.) used by every athlete no matter professional or not.



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CHAPTER 1

BCAA IPH AEN AMINO ACID PEPTIDE COMPLEX

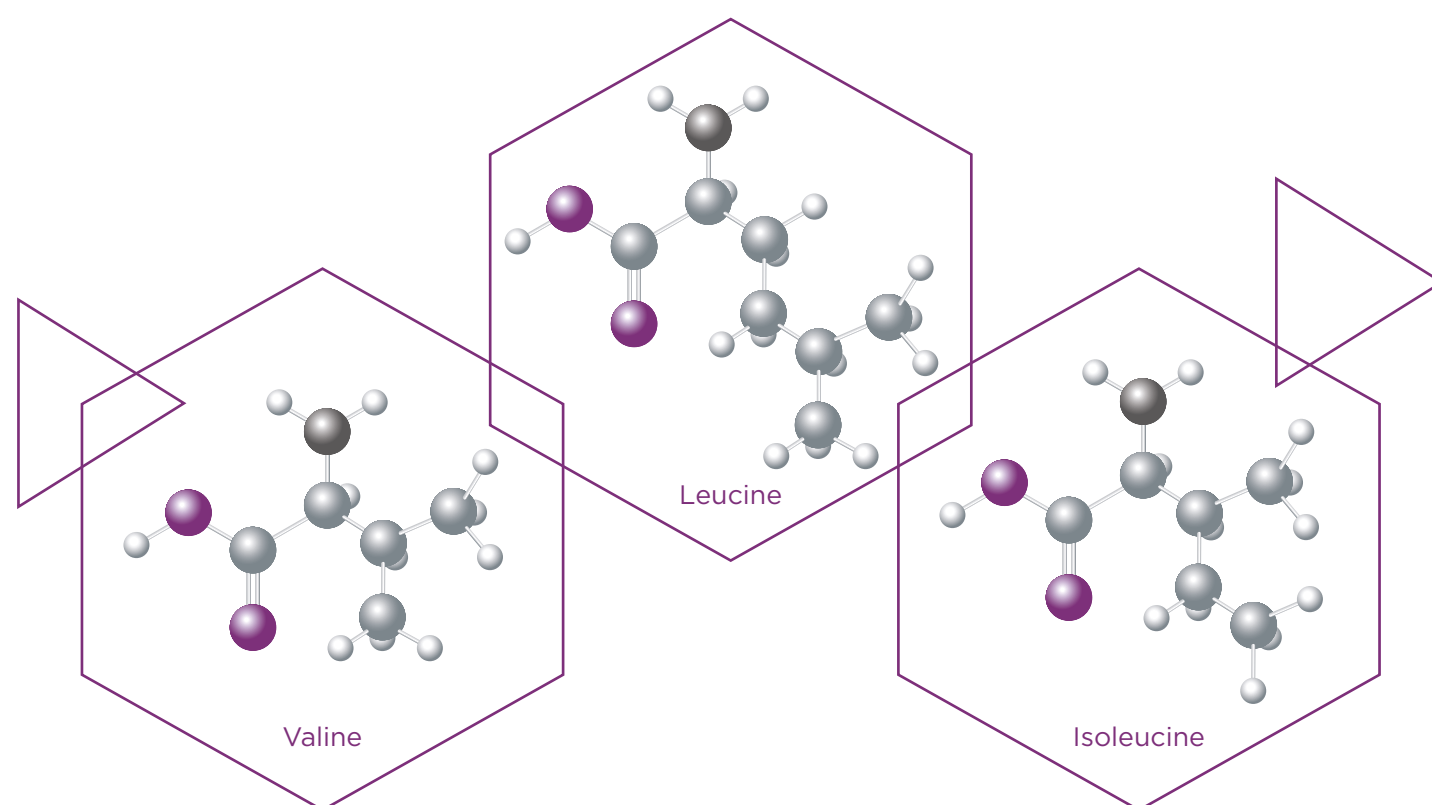
BCAA IPH AEN AMINO ACID PEPTIDE COMPLEX

BCAA IPH AEN is an innovative product consisting of a BCAA complex and of the IPH AEN peptide

At the heart of the innovative product there lies a BCAA complex which has already proven effective among professional athletes. It consists of three essential amino acids: leucine, isoleucine, valine.

The amino acid complex accelerates gain of dry muscle mass. Leucine, valine and isoleucine cannot be produced by our body and thus should be supplied externally.

The product contains a well-balanced set of amino acids, is easily absorbed by the body and acts at a molecular level.



BCAA health properties

1. Improvement of strength indicators and a source of energy.

Amino acids help save protein in muscles which is heavily consumed during intensive workouts. BCAA complex helps stop the process of catabolism and contributes to the recovery of the muscles damaged at training. Besides leucine together with isoleucine provide the body with energy which is indispensable for productive physical activity.

Combination of BCAA with the short peptide IPH AEN in one complex provides additional protection to joints the cartilaginous tissues of which are intensively used during the training process.

2. Accelerated gain of dry muscle mass.

All the three amino acids contribute to the synthesis of tissues and are indispensable for gaining the muscle protein. Essential amino acids are the result of proteolytic cleavage of protein and thus contribute to rapid muscle growth.

3. Insulin synthesis and production of growth hormone.

The product has a positive impact on the production of the growth hormone and insulin which are indispensable for the normal muscle growth, recovery and its functioning. Moreover amino acids contribute to the growth hormone and insulin balance.

4. Fat burning.

Consumption of the BCAA complex increases the level of leptin responsible for appetite, fat burning and energy consumption.



IPH AEN peptide complex

The BCAA complex is enriched with the short peptide IPH AEN

Peptides just like proteins are composed of chains of amino acids. Unlike proteins the size of peptides does not exceed 1nm which is why they belong to the nano world. They can consist of more than 10-20 chains (polypeptides) but short peptides are composed of only 3-4 amino acids forming peptide bonds and are “data carriers”.

Peptides send a specific signal from one cell to another in order to provide good functioning of the body. If a cell functions well, so does the whole body organ or system. Globally peptides impact many functions of the body and their action depends on which group they belong to.

The short peptide IPH AEN is a peptide responsible for production of cartilaginous tissues and good functioning of the joints.

IPH AEN health properties

1. Maintaining full functionality of joints;
2. Synthesis of cartilaginous tissues and effective regeneration of the bone tissue;
3. Counteraction of the degenerative processes in the cartilage tissue of the joints;
4. Joint disease prevention

Harvard University conducted a large-scale study, which proved that milk is not the source of useful micronutrients for the health of joints and bones. The highest degree of development of osteoporosis is among people who regularly drink milk.

The IPH AEN peptide complex together with well-balanced healthy food help reduce the impact of factors contributing to the appearance of degenerative processes in the joints.

Results

The BCAA amino acid complex together with the short peptide IPH AEN improve strength indicators and body endurance, increase muscle growth and help protect the joints during intensive physical activity.

The short peptide IPH AEN is quickly absorbed into the blood system and activates important physiological processes.

The BCAA enriched with the IPH AEN peptide is an innovative product with biological activity which is easily absorbed, has no side effects and is absolutely safe. It doesn't contain any conservatives or other harmful additives.

IPH AEN

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CHAPTER 2

IPH AEN RESEARCH RESULTS

STUDY OF BIOLOGICAL ACTIVITY
OF THE IPH AEN PEPTIDE
COMPLEX IN RAT CHONDROCYTE
CULTURES

BCAA IPH AEN AMINO ACID PEPTIDE COMPLEX

Objective of the study

Objective of the study

The objective of the experiment was to study chondroprotective properties of a peptide with the conventional name of IPH AEN in cultures of rat cartilaginous tissues.

Tasks:

1. To study the effect of IPH AEN on the expression of Ki67 (proliferation) and p53 (apoptosis) markers in "young" ("m") and "old" ("c") rat chondrocyte cultures.
2. To study the effect of IPH AEN on the expression of the marker of remodelling of the intercellular matrix and of the activity of MMP13 chondrocytes in "m" and "c" rat chondrocyte cultures.
3. To assume the process of IPH AEN chondroprotective action.

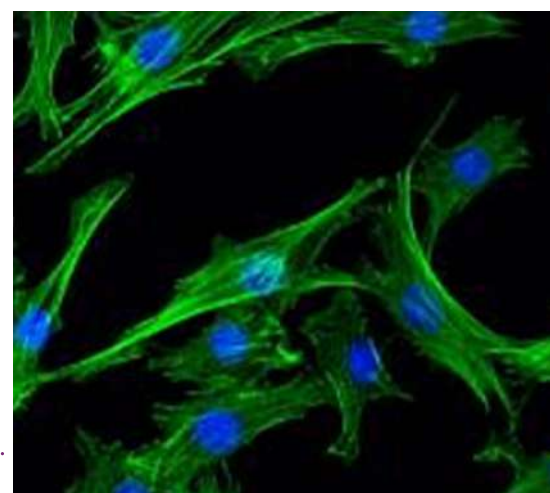


Figure 1. Primary culture of chondrocytes. Immunofluorescent confocal microscopy

Research materials and methods

The object of the study was the dissociated cultures of cartilage cells of Wistar rats, 3rd and 14th passage:

- 1 — **Control group**
pure culture
- 2 — **Group 2**
IPH AEN added (20 ng/ml);
- 3 — **Group 3**
IPH AVN added (20 ng/ml).

When studying the chondroprotective properties of IPH AEN, IPH AVN served as the negative control group. The concentration of 20 ng/ml was selected based on the literature data.

The cultures were cultivated up to the 3rd passage and up to the 14th, during which the cells were scattered on the plates and their immunocytochemical staining was performed. The 3rd passage was regarded as a "m" culture, and the 14th passage — as a "c" culture in accordance with the model of cell ageing passages.

IPH AVN and IPH AEN peptides were studied in the form of dried down powders diluted in a nutrient medium till 20ng/ml.

Chondrocyte cultures are taken from a cartilaginous tissue. The cartilage tissue was crushed and placed in a 0.2% solution of NB4 collagenase for 30 minutes at 37°C.

The cells were plated on the culture plastic without support in the DMEM/F12 growth medium enriched with a 10% bovine fetal serum (Fetal bovine serum, FBS, Autogene Bioclear), 100 U/ml penicillin (Gibco), 100 U/ml streptomycin (Gibco), 2 mmol/l L-glutamine (Invitrogen).

The medium was changed after 3 days.

The general view of rat chondrocyte cultures is shown in this figure 1.

For immunocytochemical studies of chondrocytes antibodies to Ki67 and p53 were used together with a marker for remodelling of the intercellular matrix and functioning of the MMP13 chondrocytes (1:120).

These molecules are an integral part of the functional activity of chondrocytes:

- **Ki67** is a marker for evaluating the reduction of cell proliferation and the degree of involution.
- **Protein p53** is a transcription factor, serves as a suppressor for the formation of malignant tumours (due to the activation of apoptosis in the tissues of the body). Activated by signals of disrupted cell activity or during ageing.
- **Matrix metalloproteinases (MMPs)** is a family of zinc metalloproteinases which participate in the exchange of proteins of the intercellular matrix. They have high activity with osteoarthritis.

The preparations were stained according to the standard protocol.

Research materials and methods

Statistical processing of results

Calculations included the arithmetic mean, standard deviation and confidence interval for each sample in Statistica 6.0.

The analysis of the type of distribution was determined by the Shapiro-Wilk criterion.

Student's criterion (differences in means) was used for data with a normal distribution.

To identify heterogeneous groups in significantly heterogeneous samples, multiple comparisons were performed using the Mann-Whitney U test.

The critical level of reliability of the 0-hypothesis is 0.05.

Morphometry

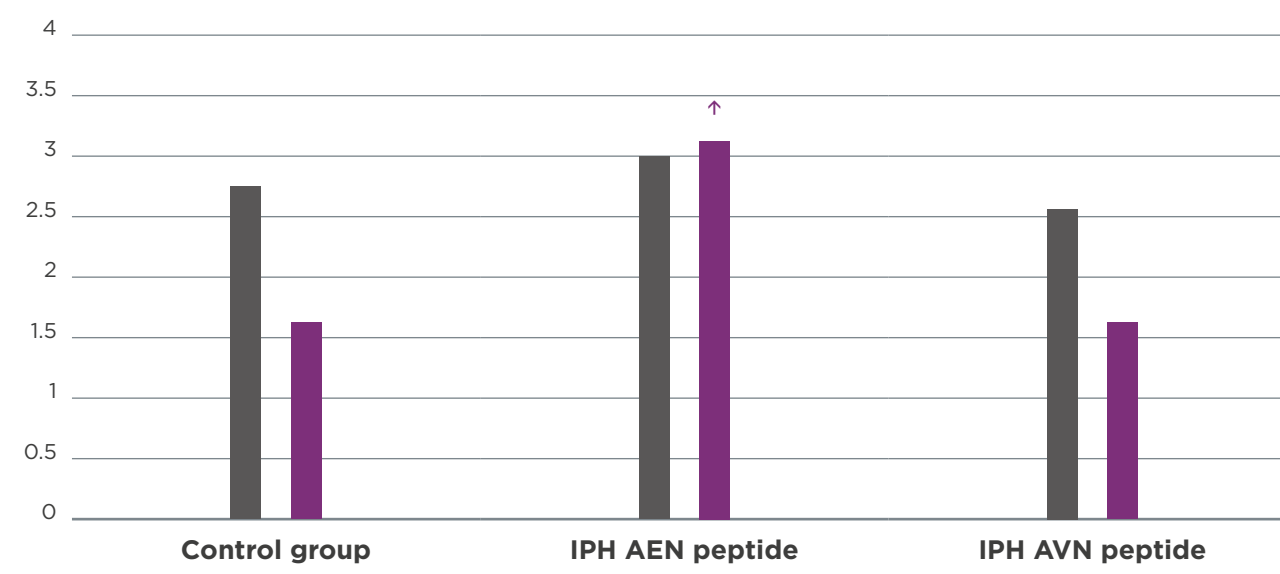
The confocal microscope Olympus FluoView 1000, Olympus FluoView ver 3.1b software was used. 10 fields of vision at $\times 200$ were analyzed.

To calculate the relative area of expression, the area of immunopositive cells was divided by the area of all cells in the field of view and expressed in % (for the MMP13 marker) and the area of immunopositive nuclei was divided by the total area of the nuclei in the field of view (for p53, Ki67 markers).

Results of the study

Effect of the peptides on Ki67 expression in the chondrocyte culture

Ki67 expression area, %



- “young” cultures
- “old” cultures

It was shown by the immunofluorescence method that the expression area of Ki67 in the control of “m” cultures ($2.8 \pm 0.3\%$) is 1.8 times greater than in “c” ($1.6 \pm 0.1\%$) cultures.

Under the action of IPH AEN, the expression of Ki67 in “c” cultures increases by 2.

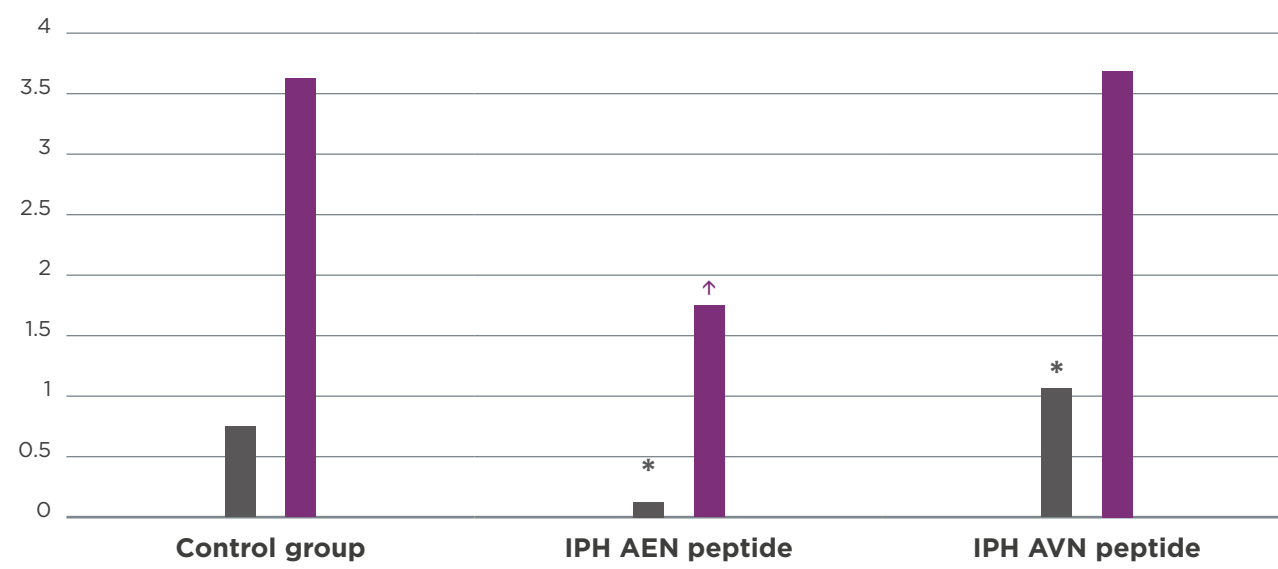
In “m” cultures, IPH AEN peptide did not significantly affect the expression of Ki67. The IPH AVN peptide did not affect the expression of Ki67 in chondrocyte cultures.

↑ — p < 0,05 in comparison to the control group “c” of the culture.

Results of the study

Expression of p53 in chondrocyte culture under the influence of peptides

p53 expression area, %



- “young” cultures
- “old” cultures

The same method showed that the expression area of p53 in the control of “m” cultures (0.8 ± 0.1%) was 4.6 times lower than in “c” (3.7 ± 0.6%).

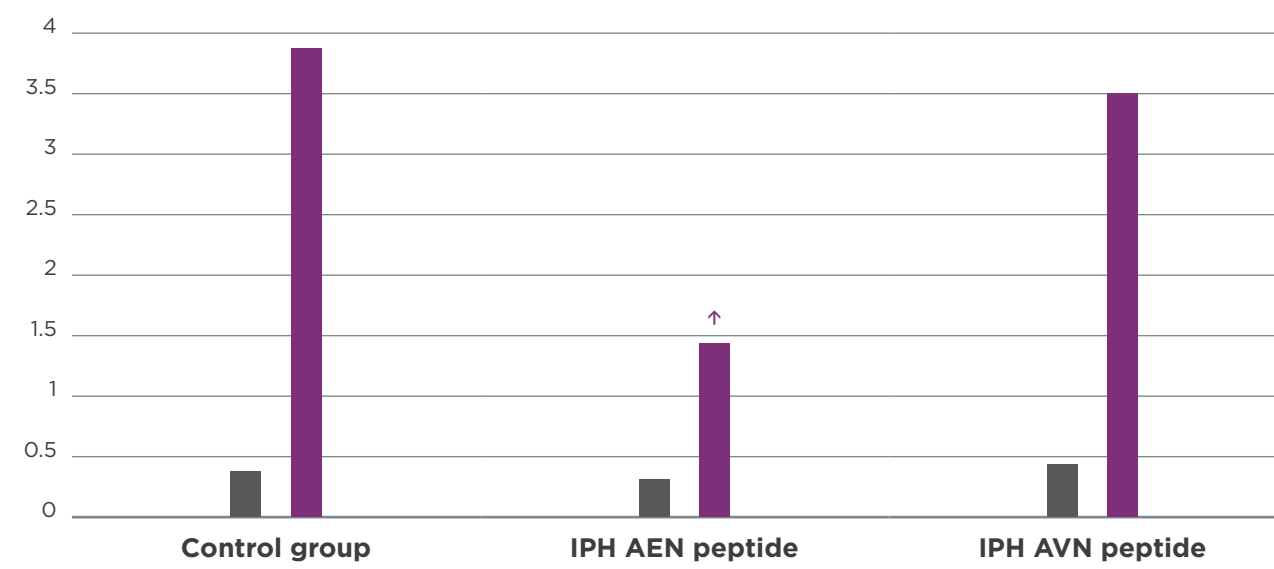
Under the influence of IPH AEN, the expression of p53 in “m” and “c” cultures decreased by 1.8 and 2.1 times.

Under the influence of IPH AVN peptide, an increase in p53 expression in “young” chondrocyte cultures was by 1.5 times. In this case, the peptide IPH AVN did not affect the studied indicator in the “old” cultures.

* – p < 0,05 in comparison to the control group “m” of the culture;
 ↑ – p < 0,05 in comparison to the control group “c” of the culture.

Effect of the peptides on the expression of MMP13 in the chondrocyte culture

MMP13 expression area, %



- “young” cultures
- “old” cultures

It was shown by the immunofluorescence method that the expression area of MMP13 in the control of “m” cultures (0.3 ± 0.05%) is 13 times lower than in “c” cultures (3.9 ± 0.7%).

Under the influence of IPH AEN, the expression of MMP13 in the “c” cultures decreased by a factor of 2.7.

The IPH AEN peptide did not affect the expression of MMP13 in “m” chondrocyte cultures.

The IPH AVN peptide did not affect the indicator in the “young” and “old” cultures of chondrocytes.

↑ – p < 0.05 in comparison to the “c” control group of the culture.

Conclusions

Data on the effect of the IPH AEN peptide on the expression of Ki67, p53, MMP13 proteins

The data on the IPH AEN effect on the expression of Ki67, p53, MMP13 proteins in chondrocyte cultures during their ageing can play an important role in understanding molecular mechanisms of action of this peptide during degenerative processes in the cartilaginous tissue of the joints.

Ageing activates apoptosis processes (p53 expression) while proliferation processes (Ki67 expression) are being slowed down.

This is one of the factors of the development of pathology of the musculoskeletal system during ageing and with people having physical activities.

The same is observed with ageing of chondrocytes in the experiment. The IPH AEN peptide stimulates proliferation of chondrocytes and reduces the intensity of apoptosis under cell ageing conditions.

The IPH AEN peptide also reduces the expression of MMP13, which is typical of inflammatory diseases of the cartilage tissue. Thus, the IPH AEN peptide can be considered as a substance which is potentially promising as a chondroprotector.

Conclusions

1. During ageing of chondrocytes in the culture, the amount of Ki67 decreases by 1.8 times. IPH AEN increases the expression of the proliferative protein Ki67 in "m" and "c" chondrocyte cultures by 1.7 and 2 times. With ageing of chondrocytes in the culture, production of p53 increases by 4.6 times. The IPH AVN peptide reduces the expression of p53 in "m" and "c" cell cultures by 1.8 and 2.1 times.
2. During ageing of chondrocytes in the culture, the expression of MMP13 increases by 13 times. Under the action of the IPH AEN peptide, the expression of MMP13 in "c" cultures decreases by a factor of 2.7.
3. Proliferation stimulation (expression of Ki67 protein), reduction in apoptosis (p53 protein) and remodelling of the intercellular matrix (expression of MMP13) by the IPH AEN peptide may indicate the ability of this peptide to prevent the development of cartilage degeneration of the joints.

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CHAPTER 3.1

RESEARCH RESULTS OF THE BCAA IPH AEN COMPLEX

1 / EFFECT OF THE" SPORTS
SUPPLEMENT BCAA 2:1:1 + IPH AEN
PEPTIDE COMPLEX ON MUSCULAR
STRENGTH AND PHYSICAL FATIGUE
OF MICE

BCAA IPH AEN AMINO ACID PEPTIDE COMPLEX

Research materials and methods

Objective of the study

The purpose of the study was to study the effect of sports nutrition BCAA 2:1:1 + Peptide complex IPH AEN on muscle mass, recovery and endurance of experimental mice.

Research materials and methods

240 female and 240 male CBA mice were selected for the study. The animals were 2 months old of an average weight of 18-20g. The animals were kept in polypropylene (30 × 21 × 9 cm) cells, 10 mice per 1 cell with an average temperature of 22 ± 2°C. Animals had unlimited access to food and water.

The mice were divided into 3 groups of 160 per each (equal proportion of males and females):

- 1 **The control group**
(intact animals)
- 2 **The comparison group**
9 mg BCAA 2:2:1 sports nutrition supplement per each mouse in addition to their daily diet for 6 months
- 3 **The main group**
9 mg BCAA 2:1:1 + IPH AEN PEPTIDE COMPLEX sports nutrition supplement per each mouse in addition to their daily diet for 6 months

Calculation of the dosage was based on BCAA recommendations for use: 10 g per serving 3 times a day — a total of 30g per day for a person weighing 70 kg, or 0.43g per 1 kg of body mass, or 9mg per mouse. Each animal was weighed once a month.

The following indicators were calculated for each individual group:

- average body weight and the standard error of mean;
- indicators of a linear regression of weight increase with age and their average value by group.

After 9 months of observation, the mice were classified as follows:

- Mice with a low body mass index (<29 g)
- Mice with an average body mass index (29–33 g)
- Mice with a high body mass index (>33 g)

Every 9th, 12th and 15th month of the experiment the number of mice with different body mass index (in %) in each observed group was recorded.

Study of muscle strength and physical fatigue

Muscle strength and fatigue were measured 6 months into the experiment.

The protocol:

- The mice were suspended on a rope at 75-80cm of height, so that they would hold to the rope with the front paws until getting tired and falling down.
- The time extend after which the mice would stop holding on to the rope and would actually fall down was registered as the hanging time.
- 20 minutes later the same experiment was repeated.
- The two suspension time indicators were expressed in seconds in order to calculate the mean, the sum and the difference between the time of the first and second hanging period, which would demonstrate the mice's muscle strength recovery.
- The ratios between the weight of the mouse to the time of its first and second hanging period as well as the hanging time to the mass of the animal were calculated in regards to the weight of the animal. Then the searchers found the average value, the sum and the difference of these indicators. The same indicators were calculated per each group and per each class, depending on the body mass index of the animal.

The animals were examined during 15 months.

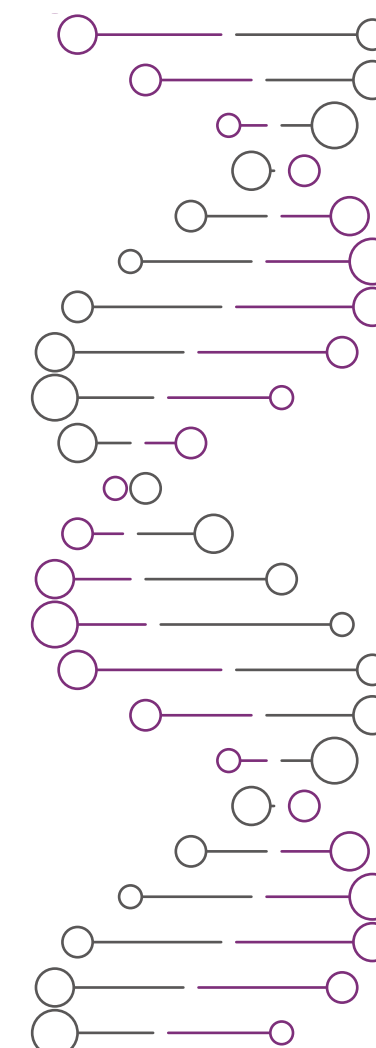
BCAA IPH AEN AMINO ACID PEPTIDE COMPLEX

Statistical processing of research results

The processing of the study statistical results was done with the STATGRAPH statistical software package.

The parameters of the regression equation for the graph of age dynamics of body weight were calculated with the help of variational statistics.

The certainty value was verified with Student's t-test, Fisher's precision method, X2, Mann-Whitney-Wilcoxon U-test for heterogeneous data.



Results of the study

Change in body weight during ageing

The weight of 15-month-old mice exceeded the weight of 6 month-old mice by 50% in the control group, by 55% in group 2, by 56% in group 3 (p <0.05 in group 2 and 3 with respect to the control group) (Table 1).

The age-related dynamics of the mice's body weight (in grams) which received the dietary supplement BCAA 2:1:1 and BCAA 2:1:1 + IPH AEN peptide complex

Table 1

Animal group	BODY MASS INDEX (g)				
	Age (months)				
	2	6	9	12	15
Control	19,08 + 0,07	20,83 + 0,15	23,11 + 0,17	28,12 + 0,19	31,34 + 0,21
BCAA 2:1:1	18,67 + 0,09	21,02 + 0,14	24,16 + 0,11¹	29,36 + 0,21¹	32,68 + 0,20¹
BCAA 2:1:1 + Peptide complex IPH AEN	18,77 + 0,05	20,94 + 0,10	24,39 + 0,13¹	29,42 + 0,15¹	32,66 + 0,19¹

With age, the percentage of animals the mass of which was very different from the average indicator significantly increased.

¹ – p <0,05 – the difference is significant compared to the index in the control group.

Breakdown of mice by body weight in groups consuming BCAA 2:1:1 and BCAA 2:1:1 + IPH AEN Peptide complex in their daily ration

Table 2

Animal group	QUANTITY OF MICE IN CLASSES PER THEIR BODY MASS INDEX (%)		
	< 29 g	29–33 g	> 33 g
After 12 months			
Control	30,2	59,1	10,8
BCAA 2:1:1	19,1¹	63,7	17,2¹
BCAA 2:1:1 + Peptide complex IPH AEN	14,9¹ ↑	63,2	21,9¹ ↑
After 15 months			
Control	18,2	72,1	9,7
BCAA 2:1:1	8,9¹	54,3¹	36,8¹
BCAA 2:1:1 + Peptide complex IPH AEN	7,6¹ ↑	51,0¹	41,4¹ ↑

During the entire experiment, the prevailing weight class was the intermediate one (51-72% in all groups).

The number of animals with a low body mass index was smaller, however bigger in group 2 and 3 with a high body mass index than in the control group.

This difference is most obvious on the 15th month of the experiment, when the percentage of mice with an average body mass index

in the intact group remained at around 72.1, and in the groups receiving BCAA, it decreased to 54.3 and 51 respectively in groups 2 and 3.

In experimental groups the number of mice with a high body mass index is 4 times greater than their number in the control group (36.8% and 41.4% in group 2 and 3, versus 9.7% in the control, p <0.05).

¹ – p <0,05 – the difference is significant compared to the index in the control group;

↑ – p <0,05 – the difference is reliable compared to the index in the group of mice receiving BCAA.

Results of the study

The effect of the sports nutrition products BCAA 2:1:1, BCAA 2:1:1 + IPH AEN PEPTIDE COMPLEX

Among the mice with a high body mass index from group 3, the time of the first and the second suspensions is significantly greater ($142.39 \pm 13.43s$, $155.16 \pm 17.54s$, $p < 0.05$) than among the mice from group 2 ($119,67 \pm 13.45s$, $128.43 \pm 16.11s$, $p < 0.05$).

The animals with a high body mass index from the control group fell down almost immediately ($12.41 \pm 2.17s$, $15.78 \pm 3.11s$).

Fatigability and the possibility of recovery were determined by the difference in the time of the first and second suspensions.

It was measured for animals with a high body mass index.

For the mice from group 3 receiving BCAA with the IPH AEN peptide, this difference was of $12.77 \pm 1.31s$, for the mice from group 2 receiving BCAA without peptides, it was of $8.76 \pm 1.2s$ ($p < 0,05$), animals from the control group showed an indicator of $3.37 \pm 1.56s$ ($p < 0.05$).

Conclusions

1. The increase in the muscular mass of the mice from group 3 was due to the intake of BCAA with the peptide, which increased muscle strength and reduced fatigue of the mice.
2. BCAA 2: 1: 1 + IPH AEN Peptide complex contributed to a rapid recovery of 1.46 times greater than the BCAA without peptides, and by 3.8 times more than in the control group.

BCAA IPH AEN AMINO ACID PEPTIDE COMPLEX

IDEAL PHARMA PEPTIDE

CHAPTER 3.2

RESEARCH RESULTS OF THE BCAA IPH AEN COMPLEX

2 / EVALUATION OF THE EFFICACY
FROM APPLICATION OF THE SPORTS
NUTRITION PRODUCT BCAA 2: 1: 1 +
IPH AEN PEPTIDE COMPLEX
IN THE TRAINING PROCESS
OF PROFESSIONAL AND RECREATIONAL
ATHLETES

BCAA IPH AEN AMINO ACID PEPTIDE COMPLEX

Objective of the study

Under intense physical activity, the cartilaginous surface suffer microtraumas and strain injuries, which gradually leads to osteoarthritis. Post-traumatic chondropathy is the cause of degenerative-dystrophic joint injuries, so the prevention of such a disease in order to reduce the risk of a developing osteoarthritis is an urgent problem.

Objective of the study

The objective of this work is to study the efficacy of application of the sports nutrition product BCAA 2:1:1 + IPH AEN Peptide complex in the training process of professional and recreational athletes. The previous studies demonstrated the chondroprotective effect of the IPH AEN, which suggests that it is efficacious in preventing osteoarthritis and correcting the developing chondropathy.

Tasks of the study

1. To study the effect of BCAA 2:1:1 + IPH AEN Peptide complex administration course (10 g 3 times a day for 60 days) on the functional activity of the knee joint of professional sportsmen engaged in team sports. To evaluate the expediency of using the product with the IPH AEN peptide in the training process of volleyball players.
2. To evaluate the effect of the tested product containing the IPH AEN peptide (10 g twice a day in the morning and evening for 60 days) the indices of the functional activity of the knee joint of men doing fitness and draw conclusions on the advisability of its use.

Characteristics of participants

Professional volleyball players and recreational athletes participated in the study (working out 3 times a week). All the tested subjects had post-traumatic chondropathy of the knee joint of 1st-2nd degree in their medical history.

45 men aged 18-40 years old took part in the experiment. They were divided into 2 groups:

The first group — professional athletes

18 volleyball players (Candidates in Master of Sports) aged 18 to 38 years (26.8 ± 3.4 years). Randomly, they were divided into 2 subgroups of 9 persons — the main and the control groups.

1 (MAIN) SUBGROUP

9 people — 60 days received BCAA 2: 1: 1 + IPH AEN Peptide complex at a concentration of 10 g per person, 3 times a day.

2 (CONTROL) SUBGROUP

9 people — received BCAA 2:1:1 according to the same scheme.

The supervision of the course administration was ensured by a doctor. All athletes had the same conditions (food, medical control, living and training).

The second group — recreational athletes

27 men aged 21-40 years old (28.6 ± 2.9 years old) attending fitness classes 3 times a week for 1.5 hours.

They were also divided into 2 subgroups: the main group (17 persons) and the control group (10 persons).

1 (MAIN) SUBGROUP

17 people — received BCAA 2:1:1 + IPH AEN Peptide complex at a concentration of 10 g twice per day for 60 days.

2 (CONTROL) SUBGROUP

10 people — received BCAA without peptides according to the same scheme.

Methods of research

The volunteers were examined according to the following criteria

- Severity of the pain syndrome based on the Visual Analogue Scale (VAS).**
- The function of knee joints when walking on an even surface for 30 m.**
- The state of the knee joint according to the KOOS scale (Sweden):** "Pain", "Symptoms", "Complexity of performing household activities", "Sport, activity, rest", "Quality of life". 100 points — no symptoms, 0 — pronounced.
- The state of the cartilage: ultrasonography (Samsung SonoAce R3) with a high-frequency linear sensor (7.5 — 10.0 MHz). It tested the state of the hyaline cartilage of the condyles of the femoral bone of the injured knee joint of the Cartilage Degeneration Index(CDI).**

The level of cartilage changes was assessed according to the ICRS classification for ultrasonic diagnostics:

- 0 — normal cartilage;
- 1 — increased echogenicity, slightly uneven contour;
- 2 — increased echogenicity, heterogeneous structure, uneven contour, thinning of cartilage by up to 50%, CDI 0.5-0.8;
- 3 — increased echogenicity, heterogeneous structure, strongly uneven contour with erosions, thinning of cartilage by more than 50%, CDI less than 0.5;
- 4 — large areas of absence of cartilage.

All parameters were evaluated before the experiment and after it was completed.

The statistical processing corresponded to standard methods for processing the results of biomedical studies (Student's t-test, Spearman's correlation analysis and factor analysis by the principal components method) in Statistica 5.5.

Results of the study

Intensity of pain in the knee joint according to the Visual Analogue Scale (VAS)

Analysis of subjective evaluation of pain by the participants:

- The initial level of pain among 5 professional athletes (27.8%) and 5 recreational athletes (18.5%) was 6-8 cm ("severe pain"); 10 sportsmen (55.5%) and 18 recreational athletes (66.7%) — 4-6 cm ("moderate pain"); 3 professional athletes (16.7%) and 4 recreational athletes (14.8%) — 2-4 cm ("weak pain").
- After the end of the course of administration of the sports nutrition product with the IPH AEN peptide, the pain level was not higher than 2-4 cm, and more than 55% of professional and recreational athletes from the main subgroups noted the absence of pain.
- The indices in the subgroups taking BCAA without peptides did not change in comparison to the initial data.

Table 1

Effect of the sports nutrition products on the intensity indices of pain according to VAS

INTENSITY OF PAIN ACCORDING TO VAS, CM	PROFESSIONAL ATHLETES n = 18			RECREATIONAL ATHLETES n = 27		
	Initially	BCAA n = 9	BCAA + IPH AEN n = 9	Initially	BCAA n = 10	BCAA + IPH AEN n = 17
		60 days after	60 days after		60 days after	60 days after
0-2	0	0	5 (55,6 %)	0	0	11 (64,7 %)
2-4	3 (16,7 %)	2 (22,2 %)	4 (44,4 %)	4 (14,8 %)	3 (30,0 %)	6 (35,3 %)
4-6	10 (55,5 %)	6 (67,7 %)	0	18 (66,7 %)	5 (50,0 %)	0
6-8	5 (27,8 %)	1 (11,1 %)	0	5 (18,5 %)	2 (20,0 %)	0
8-10	0	0	0	0	0	0

Results of the study

Dynamics of functional activity of the knee joint

Initially, more than 80% of the tested subjects covered 30m in more than 30s, when the norm was no more than 20s (Table 2).

After the course of administration of the IPH AEN peptide, the observed main subgroups had positive dynamics of the time needed to cover the distance.

Among professional and recreational athletes, no one showed more than 40 seconds; about 90% of the tested subjects demonstrated the result of less than 30 seconds, 44.4% of professional athletes and 29.4% of recreational athletes covered this distance in less than 20 seconds.

In the subgroups taking BCAA without IPH AEN, 10-20% of the volunteers showed the time not different from the initial one.

Dynamics of KOOS scale indicators

The indicators "Pain", "Symptoms", "Sports Activity" and "Quality of Life" in the subgroups taking BCAA with the peptide, improved by 1.2-1.3 times compared to the initial data.

In the subgroups taking BCAA without peptides, the final indices did not differ from the initial ones.

The indicator "Daily Activity" was initially high, so its intensity did not change.

Table 2

Effect of the sports nutrition products on the time needed for covering a 30-meter distance

TIME FOR COVERING 30M, SEC	PROFESSIONAL ATHLETES n = 18			RECREATIONAL ATHLETES n = 27		
	Initially	BCAA n = 9	BCAA + IPH AEN n = 9	Initially	BCAA n = 10	BCAA + IPH AEN n = 17
		60 days after	60 days after		60 days after	60 days after
Less than 20	0	0	4 (44,4 %)	0	0	5 (29,4 %)
20-30	2 (11,1 %)	3 (33,3 %)	4 (44,4 %)	5 (18,5 %)	3 (30,0 %)	10 (58,8 %)
30-40	11 (61,1 %)	5 (55,6 %)	1 (11,1 %)	16 (59,3 %)	5 (50,0 %)	2 (11,8 %)
Over 40	5 (27,8 %)	1 (11,1 %)	0	6 (22,2 %)	2 (20,0 %)	0

Table 3

Effect of the sports nutrition products on KOOS scores

KOOS SCALE PARAMETERS	PROFESSIONAL ATHLETES n = 18			RECREATIONAL ATHLETES n = 27		
	Initially	BCAA n = 9	BCAA + IPH AEN n = 9	Initially	BCAA n = 10	BCAA + IPH AEN n = 17
		60 days after	60 days after		60 days after	60 days after
Pain	72,6 ± 9,8	75,3 ± 11,2	85,2 ± 9,4¹	74,2 ± 11,6	76,1 ± 12,4	83,3 ± 10,1¹
Symptoms	62,7 ± 8,4	67,7 ± 10,0	75,4 ± 11,6¹	60,3 ± 9,9	65,4 ± 11,6	74,7 ± 9,4¹
Daily activity	82,5 ± 10,2	86,3 ± 8,9	85,7 ± 8,8	83,7 ± 10,3	85,1 ± 12,8	86,3 ± 10,6
Sports activity	60,5 ± 10,6	65,2 ± 11,5	78,8 ± 9,5¹	62,1 ± 10,4	66,7 ± 12,4	76,1 ± 8,7¹
Quality of life	63,7 ± 11,1	68,5 ± 9,6	79,3 ± 8,9¹	64,2 ± 11,2	67,2 ± 10,1	78,4 ± 9,2¹

¹ – the differences are significant compared to the initial index in the relevant group.

Results of the study

Dynamics of hyaline cartilage

Before administration of BCAA with the IPH AEN peptide, professional and recreational athletes had chondropathy of 1st – 2nd degree (increased echogenicity, contour irregularities, reduced cartilage thickness, CDI 0.62-0.64) (Table 4).

Sixty days after the beginning of the BCAA course containing the peptide in the main subgroups it was observed that echogenicity decreased, the sharpness of the cartilage contour improved, the CDI index decreased by 0.04-0.05, which indicates normalization of the cartilage tissue state.

Table 4

Effect of the sports nutrition products on the index of degenerative thinning of cartilage

TESTED SUBJECTS	CDI (MEDIAL CONDYLE), STANDARD UNIT		CDI (ECTOCONDYLE), STANDARD UNIT	
	Initially	60 days after	Initially	60 days after
Professional athletes BCAA	0,64	0,65	0,62	0,62
Professional athletes BCAA + IPH AEN	0,63	0,68	0,61	0,66
Recreational athletes BCAA	0,62	0,62	0,61	0,60
Recreational athletes BCAA + IPH AEN	0,64	0,68	0,63	0,67

In groups taking BCAA without peptides, the results of the ultrasound study did not change.

Conclusions

1. In the presence of chondropathy of 1st-2nd degree among professional and recreational athletes, the use of the sports nutrition supplement BCAA 2:1:1 + IPH AEN Peptide complex during 60 days leads to a significant improvement of the KOOS test parameters "Pain", "Symptoms", "Sports Activity", "Quality of life".
2. VAS parameters are also significantly improved – reduced pain, reduced time of walking the distance of 30m.
3. Positive dynamics of the structure of hyaline cartilage was observed.
4. The product has a positive effect on the state of the joints among people subject to physical stress. Since cartilage tissue requires much time to influence its functional state, it is recommended to have a prolonged course of administration of BCAA 2:1:1 + IPH AEN Peptide complex for people doing sports or having any other types of intensive physical activities in order to prevent progression and stop pathological processes in the joints.



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