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—
RELEVANT RESEARCH

BCAA IPH AVN 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.

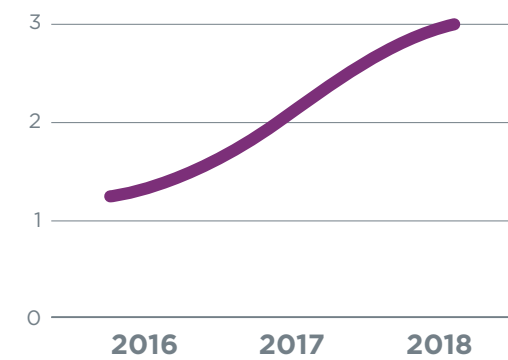
Investing in innovative products is the best decision to obtain economic growth and maximize profit of your company. Standard products presuppose high competition and therefore low added value for the manufacturing company.

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 \$



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.

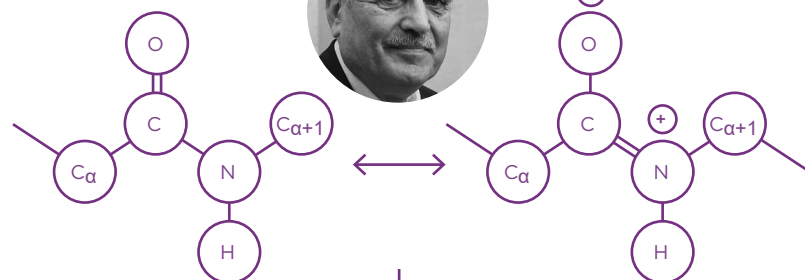
Peptide History

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...

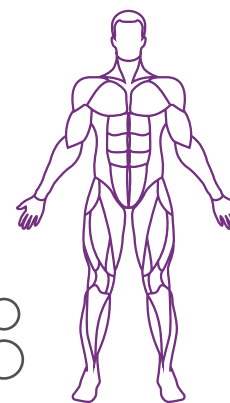


Once upon a time...



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.



Recently...



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.

Today...



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.

Tomorrow...

IDEAL PHARMA PEPTIDE

CHAPTER 1

BCAA IPH AVN AMINO ACID PEPTIDE COMPLEX

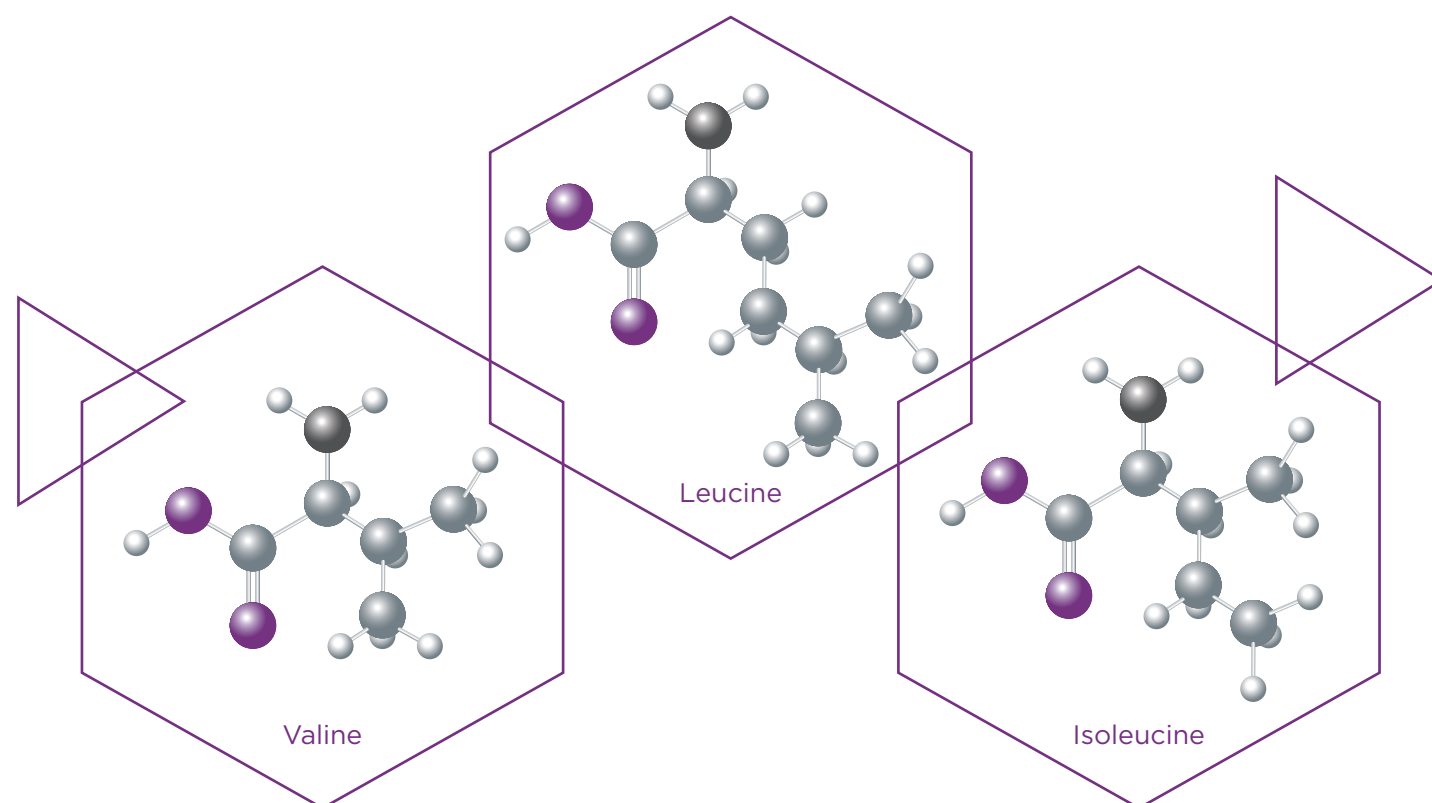
BCAA IPH AVN AMINO ACID PEPTIDE COMPLEX

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

At the heart of the innovative product there lies a BCAA complex which 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 is easily absorbed by the body, acts at the molecular level and creates favourable conditions for dry muscle gain.



BCAA health properties

1. Retaining and improvement of dry muscle gain.

All the three amino acids are the key building material for the muscle tissues. Intake of the amino acid complex BCAA IPH AVN stimulates muscle gain even on a low protein diet.

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

Intensive workouts consume a lot of amino acids in the body which reclaims them back from the stock in the muscle tissues to make up for the loss. 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 by providing the necessary amino acids. Moreover leucine and isoleucine provide the body with energy which is indispensable for a productive physical activity.

3. Fat burning.

Consumption of the BCAA complex influences the level of leptin responsible for the metabolic processes in the body. Leucine and isoleucine have a positive impact on the energy consumption, suppress appetite and contribute to elimination of subcutaneous fat.

4. Insulin synthesis and production of growth hormone.

Consumption of the product increases the level of glutamine which is indispensable for the production of the growth hormone. The amino acid complex provides the body with the elements for building new proteins, contributes to the production of insulin which transports all nutrients to the muscle tissues and thus accelerates muscle growth.



IPH AVN Peptide Complex

The innovative BCAA complex is enriched with the short peptide IPH AVN

Peptides are physically active compounds having the same structure as proteins. Unlike proteins the size of the peptide molecule is much smaller which is why they belong to the nano world.

They can contain more than 20 amino acids (polypeptides), but short peptides contain only 3-4 amino acids forming peptide bonds. Short communication makes them more efficacious and safe.

Peptides influence multiple functions of the body from geno-regulation to toxin elimination from the body. Their action depends on which group they belong to. The short peptide IPH AVN is a peptide of the vascular system.



Properties of the IPH AVN peptide complex

1. Provides protection of the blood vessels.
2. Increases stability of blood vessels during intensive workouts.
3. Has a preventive effect by reducing the risk of developing cardiovascular pathologies.
4. Slows down ageing processes of the body and in particular the process of losing elasticity of the vascular endothelium.
5. Reduces the influence of negative factors of the environment on the cardiovascular system.

It has been scientifically proven that regular consumption of products of animal origin can become the cause of certain heart conditions. Such food makes our blood viscous, changes the endocrine profile and increases the cholesterol level.

The short peptide IPH AVN counteracts this process and protects the body from the adverse effects of harmful food. Healthy food in combination with the IPH AVN peptide is the optimal solution for the preventing "incurable" diseases of the cardiovascular system.

Results

BCAA amino acid complex in combination with the IPH AVN peptide increases endurance of the body, helps ensure normal muscle growth and protects blood vessels during and after intensive training.

The IPH AVN peptide, being a short peptide, is fully absorbed by the body and is quickly transferred to the circulatory system. The innovative product does not contain preservatives and other harmful additives.

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CHAPTER 2

IPH AVN RESEARCH RESULTS

STUDY OF THE BIOLOGICAL
ACTIVITY OF THE IPH AVN
PEPTIDE COMPLEX IN RAT
ENDOTHELIOCYTE CULTURES

BCAA IPH AVN AMINO ACID PEPTIDE COMPLEX

Objective of the study

Objective of the study

The objective of this research is to study the angioprotective properties of a peptide under the conventional name of IPH AVN in rat endothelial cultures.

To achieve this objective, the following tasks were set:

1. To study the effect of IPH AVN on the expression of the Ki67 marker of proliferation and the p53 marker of apoptosis in "young" and "old" rat endotheliocyte cultures.
2. To investigate the effect of the IPH AVN peptide on the expression of the VEGF angiogenesis marker and the Cx43 intercellular contact marker in "young" and "old" rat endotheliocyte cultures;
3. To identify the alleged mechanisms of vasoprotective action of the IPH AVN peptide.

Research materials and methods

Animal group

Primary cultures of vascular cells of young Wistar rats were taken as object of the morpho-functional study.

These animals were kept in animal quarters at room temperature with a 12-hour alternation of light and darkness, with unlimited access to water and food, on a normal diet for laboratory animals in accordance with their containment standards.

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

For most of the dissociated cell cultures, the optimal peptide concentration is 20 ng/ml.

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 "young" culture, and the 14th passage — as an "old" culture in accordance with the model of cell ageing passages.

The IPH AEN served as a negative control group to study angioprotective properties of the peptide with the conditional name of IPH AVN.

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

Research materials and methods

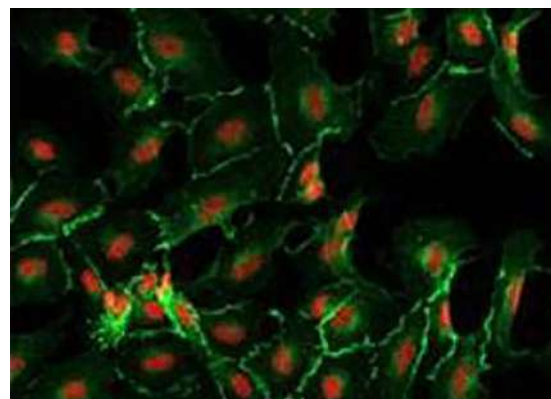
Preparation of the peptide process solution

To study the angioprotective properties of peptides, IPH AVN and IPH AEN were dried down to powder and diluted in a nutrient medium till reaching the concentration of 20ng/ml.

Endothelial cultures were taken from aortic walls of Wistar rats. The isolated tissue of the vascular wall 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 every 3 days.

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



For immunocytochemical studies of chondrocytes primary antibodies Ki67 (1:50), p53 (1:100), VEGF (1:50) and Cx43 1:100) were used.

These antibodies play an important role in the functional activity and ageing of endothelial cells:

- **protein Ki67** is a proliferation marker used for evaluating the reduction of cell proliferation and the degree of involution in the tested organ;
- **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). It is activated by signals of disrupted DNA and also under initiating agents. It can also be sign of cell ageing and its disruptive functioning.
- **VEGF** — vascular endothelial growth factor, a signalling protein, vasculogenesis and angiogenesis inducer;
- **Cx43** — connexin43 belonging to the family of connexins, components of gap junctions, which form intracellular channels and provide diffusion of low molecular compounds between neighbouring cells. Cx43 is expressed in the vascular cells components of atherosclerosis plaques including in smooth muscle cells.

Figure 1. Primary culture of endotheliocytes.
Immunofluorescent confocal microscopy

Morphometry

Confocal microscope Olympus FluoView 1000, Olympus FluoView ver 3.1b software was used. 10 fields of vision at $\times 200$ were analyzed. Relative area of expression was calculated in %.

The relative expression was estimated:

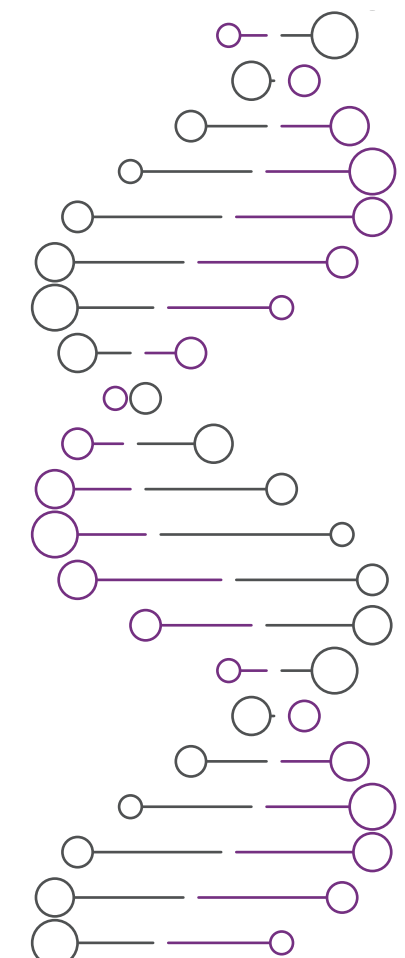
- as the ratio of the surface occupied by immunopositive cells to the general surface of cells per field of vision. This ratio is expressed in % for the marker with cytoplasmic staining (VEGF, Cx43);
- as the ratio of the surface occupied by immunopositive nuclei to the general surface of the nuclei in the field of view for markers with nuclear expression (p52, Ki67).

Statistical processing of results

Calculations included the arithmetic mean, standard deviation and confidence interval for each sample. Data processing was carried out in Statistica 6.0 software.

The analysis of the type of distribution was determined by the Shapiro-Wilk and Student's criteria (Shapiro-Wilk's W-test, Student's test).

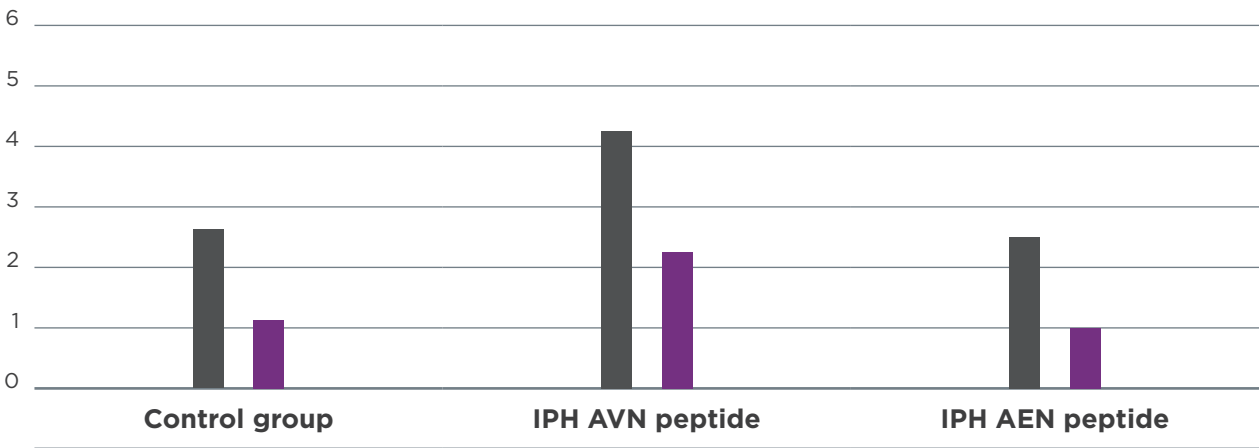
In groups with significantly heterogeneous samples, the method with multiple comparisons was performed (Mann-Whitney U-test with a critical level of the reliability of the 0-hypothesis).



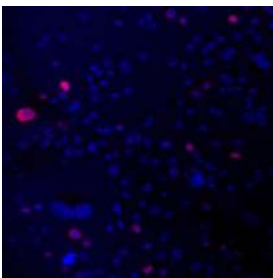
Results of the study

The effect of IPH AVN peptide on Ki67 expression in "young" and "old" cultures of rat endotheliocytes is manifested in the fact that the area of Ki67 expression in the control of "young" cultures was $(2.7 \pm 0.2)\%$, which is statistically 1.9 times greater than in "old" cultures $(1.1 \pm 0.1)\%$

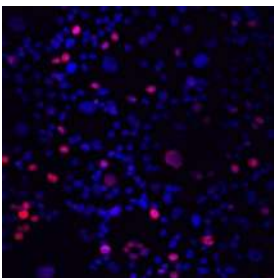
Ki67 expression surface, %



- 3rd passage of culture
- 14th passage of culture



Control group



IPH AVN peptide

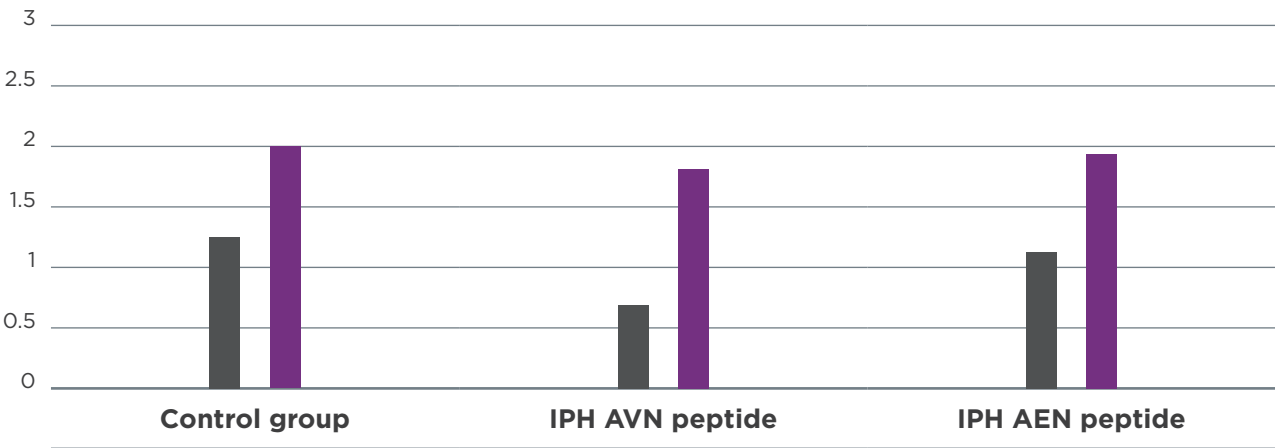
The nuclei of the cells are restained with DAPI (blue fluorescence), the expression of Ki67 is the pink glow.

Under the effect of the IPH AVN peptide, a significant increase was observed in the expression of Ki67 in "young" cultures which is by 1.7.

In the "old" cultures, the IPH AVN peptide increases the expression of Ki67 by 2. The IPH AEN peptide did not affect the expression of Ki67.

The effect of the IPH AVN peptide on the p53 expression in "young" and "old" cultures of rat endotheliocytes: the p53 expression in "young" cultures is $(1.3 \pm 0.2)\%$, and $(2.0 \pm 0.2)\%$ in "old" cultures

p53 expression surface, %



- 3rd passage of culture
- 14th passage of culture

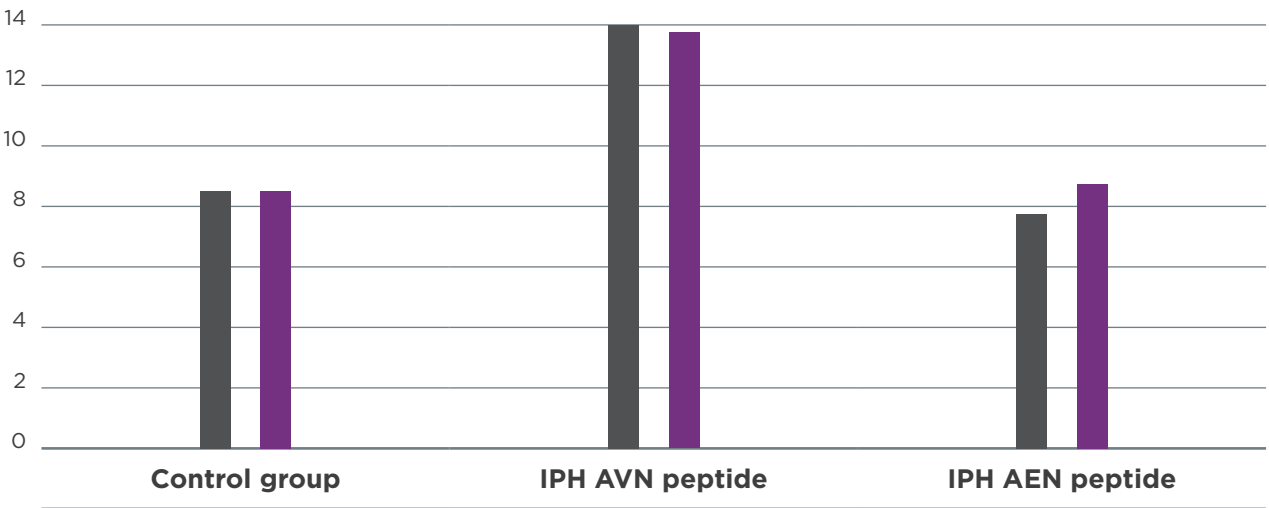
The IPH AVN peptide reduces the expression of p53 by 1.9 times, but only in "young" cell cultures.

The IPH AEN peptide did not alter the expression of this marker.

Results of the study

The effect of the IPH AVN peptide on VEGF expression in "young" and "old" cultures of rat endotheliocytes: the expression of VEGF in the control of "young" and "old" endothelial cultures remained the same ($8.5 \pm 0.1\%$)

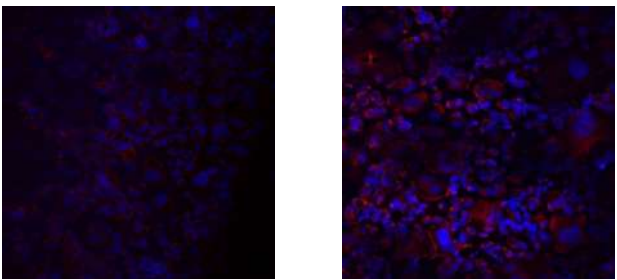
VEGF expression surface, %



- 3rd passage of culture
- 14th passage of culture

The IPH AVN peptide increases the area of expression of the marker in both "young" and "old" cultures by 1.7 and by 1.6 times respectively.

The IPH AEN peptide did not affect this index

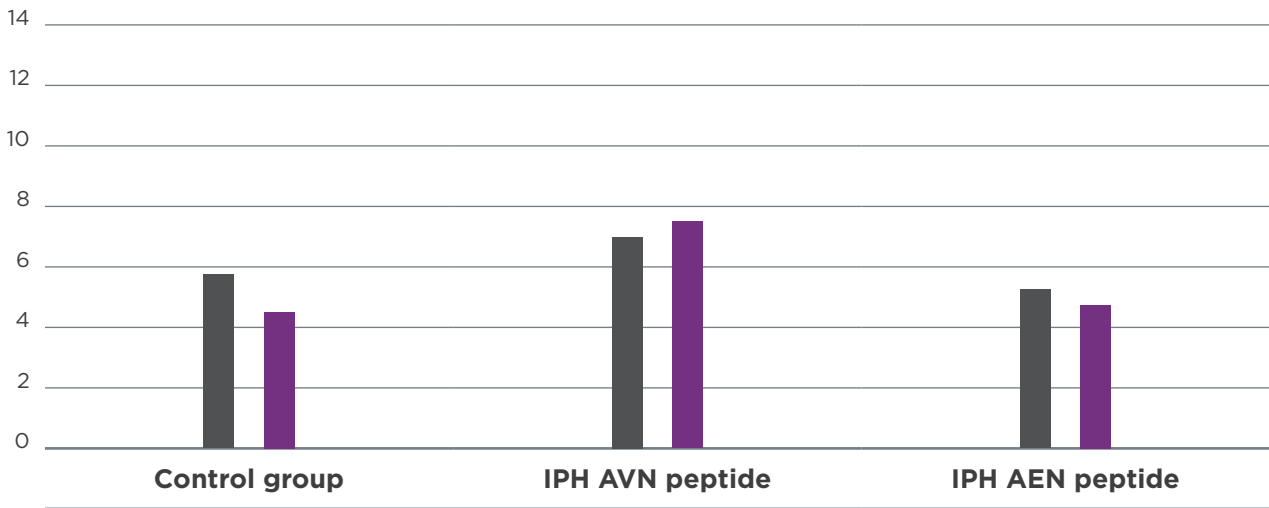


Control group IPH AVN peptide

14th passage. Confocal microscopy, x200:
Cell nuclei are stained with DAPI (blue fluorescence),
VEGF expression is the dark red glow.

The effect of the IPH AVN peptide on the Cx4 expression in "young" and "old" cultures of rat endotheliocytes: the marker expression in the control of "young" cultures was ($5.9 \pm 0.1\%$), and 1.4 times less in "old" cultures — ($4.3 \pm 0.1\%$)

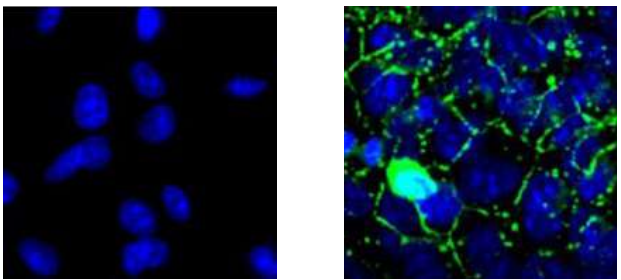
Cx43 expression surface, %



- 3rd passage of culture
- 14th passage of culture

The IPH AVN peptide extended the expression area of Cx43 in the "old" cultures by a factor of 1.7.

The peptide didn't have significant effect on expression in the "young" cultures. The IPH AEN peptide did not affect the connexin expression in any of the two cases,



Control group IPH AVN peptide

The cell nuclei are stained with DAPI (blue fluorescence),
Cx43 expression is the green glow.

Conclusions

Results of the effect of the IPH AVN peptide on the expression of Ki67, p53, VEGF, Cx43 proteins

With ageing of endothelium in a culture the expression of Ki67 decreases by 1.9 times. The IPH AVN peptide increases the expression of the Ki67 protein in "old" cultures of rat endothelial cells by a factor of 2.

With ageing of endothelial cells in a culture, the expression of p53 increases by 1.5 times. The IPH AVN peptide reduces the expression of p53 by a factor of 1.9 in "young" cell cultures.

With ageing of endothelium in a culture, the expression of the VEGF angiogenesis marker does not change. The IPH AVN peptide increases the expression of VEGF in "young" and "old" endothelial cultures respectively by 1.7 and 1.6 times.

With ageing of endothelium in a culture, the expression of the Cx43 marker of intercellular interactions decreases by a factor of 1.4. The IPH AVN peptide improves the expression of Cx43 in "old" cultures by a factor of 1.7.

Conclusions

1. The obtained data on the effect of the IPH AVN peptide on the expression of Ki67, p53, VEGF, Cx43 proteins in endothelial cultures can become important information in understanding of the molecular mechanisms of the angioprotective action of this peptide.
2. Stimulation of proliferation (expression of the Ki67 protein) and angiogenesis (expression of vascular endothelial growth factor VEGF), formation of intercellular contacts (Cx43 expression), and apoptosis decrease (p53 protein) by influence of the IPH AVN peptide indicate its ability to prevent atherosclerosis development at an early stage and protect endothelial vessels from various negative factors.

BCAA IPH AVN AMINO ACID PEPTIDE COMPLEX

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CHAPTER 3.1

RESEARCH RESULTS OF THE BCAA IPH AVN COMPLEX

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

BCAA IPH AVN AMINO ACID PEPTIDE COMPLEX

Research materials and methods

Objective of the study

The objective of the study was to find out how the peptide complex BCAA 2:1:1 + IPH AVN affects the muscular strength, fatigue and endurance of mice during 15 months.

Laboratory animals were used as research material

240 female and 240 male CBA mice were selected for the study. The animals were 2 months old of an average weight of 18-20 g. The mice were kept in cells, 10 mice per cell. The housing conditions — cell sizes, lighting and temperature — are the same. The animals were fed on all-in-one cubes and had access to tap water without restrictions.

The mice were divided into groups:

- 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 AVN 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:

30 g per day for a person weighing 70 kg, or 9 mg 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.

Methods of muscle strength and physical fatigue study

- During the study of the effect of the peptide complex BCAA 2:1:1 + IPH AVN on muscle strength and level of physical fatigue of animals mice were tested and the results were then processed.
- The mice were suspended on a rope at 75-80 cm of height, so that they would hold to the rope with the front paws until getting tired and falling down. The time of suspension was fixed. 20 minutes later the test was repeated and new data collected.
- The time was expressed in seconds and the mean of the two indicators was found as well as the sum and the difference between the time of the first and second suspension were calculated, which would account for muscle strength recovery. Additionally, the test took into account the mass of the hanging animals, which allowed to calculate the ratio of the weight of each mouse to the time of its first and second hanging and the hanging time to the mass of the animal. The average value, sum and difference of these indicators were calculated as well. The same indicators were calculated in average numbers, they helped find the sum and the difference.
- Groups held same calculations but this time by classes, depending on the body mass index of the animal.
- The animals were examined during 15 months.

Research results

Age dynamics of mice’s body index

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

Table 1 shows the results of a 15-month study of groups of animals. The data of the table describes the following picture: the body mass index increased with age and at the age of 15 months it was greater than that of 6 months by 50% in the control group, by 55% in the group with BCAA and by 55% in the group with BCAA + IPH AVN. The mice from the tested groups weighted significantly more that the control one (p<0,05).

Age dynamics of mice’s body index (measured in g) receiving BCAA 2:1:1 or BCAA 2:1:1 + IPH AVN with their daily diet

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 + 0,11 ¹	29,36 + 0,21 ¹	32,68 + 0,20 ¹
BCAA 2:1:1 + Peptide complex IPH AVN	19,02 + 0,08	21,12 + 0,11	24,31 + 0,16 ¹	29,54 + 0,18 ¹	32,72 + 0,22 ¹

As shown in the Table individual body mass index of the animals in groups vary greatly. With age the quantity of mice having the body mass index which was significantly inferior or superior to average indicators in the group was different.

¹ — p < 0,05 — the difference is reliable compared to indicators of the control group

Breakdown of mice according to body mass index per group receiving BCAA 2:1:1 or BCAA 2:1:1 + IPH AVN in their daily diet

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	19,1 ¹	63,7	17,2 ¹
BCAA 2:1:1 + Peptide complex IPH AVN	16,7 ¹ ↑	61,7	21,6 ¹ ↑
	After 15 months		
	18,2	72,1	9,7
	8,9 ¹	54,3 ¹	36,8 ¹
BCAA 2:1:1 + Peptide complex IPH AVN	7,4 ¹ ↑	51,7 ¹	40,9 ¹ ↑

During calculations of weight categories it turned out that the quantity of mice with an average weight per each age is not significantly different between groups (51 to 72%). And the number of mice with a low body mass index in all the groups was relatively smaller than in the control group.

At the same time, the number of mice with a high body mass index was greater than in the control group (Table 2). This difference was especially noticeable during the 15th month.

¹ — p < 0,05 — the difference is reliable compared to indicators of the control group;
↑ — p < 0,05 — the difference is reliable compared to the indicator in the group of mice receiving BCAA.

Research results

Effect of the sports nutrition supplements BCAA 2:1:1, BCAA 2:1:1 + IPH AVN PEPTIDE COMPLEX on muscle strength and physical fatigue of mice

The experiment showed that among the mice with increased body weight (more than 33 g) who received the sports nutrition supplement BCAA 2:1:1 + IPH AVN Peptide complex (animal group #3), the time of the first and second suspensions significantly differed from the same index in group 2, where mice received BCAA without peptides.

Thus, the animals of group 3 hung during the first suspension for 136.44 ± 17.18 sec, during the second suspension — 147.72 ± 15.33 sec, which is significantly longer than in animal group 2 which received BCAA without peptides (1st suspension — 119.67 ± 13.45 sec, 2nd suspension — 128.43 ± 16.11 sec ($p < 0.05$)).

Besides the intact mice with increased weight were unable to stay suspended on the rope and fell down almost immediately — after 12.41 ± 2.17 sec during the 1st suspension, after 15.78 ± 3.11 sec during the 2nd suspension.

The suspension time of the mice with low and medium body weight from group 3 receiving BCAA with the peptide during the first and second suspensions increased compared to both the control mice and the animals in group 2 receiving BCAA without peptides.

An important indicator is the difference between the time of the first and second suspensions, which indicates the degree of fatigue and the animals' ability to recover strength.

Thus, the mice with increased body weight who received BCAA with the IPH AVN peptide (group 3), the difference of the time between the first and second suspensions was 11.28 ± 1.21 sec, while the time difference of the mice with increased body weight, who received BCAA without peptides, was of 8.76 ± 1.2 sec ($p < 0.05$).

Conclusions

The obtained data confirm that the increased body weight of a significant part of the animals of the 3rd group who received BCAA with the IPH AVN peptide is due to the increase in muscle mass, as the BCAA 2:1:1 + IPH AVN Peptide dietary complex increased muscular strength and reduced fatigue in mice, contributing to a rapid recovery, 1.29 times faster than BCAA without peptides, and 3.35 faster than the control animals that did not receive sports nutrition at all.

BCAA IPH AVN AMINO ACID PEPTIDE COMPLEX

IDEAL PHARMA PEPTIDE

CHAPTER 3.2

RESEARCH RESULTS OF THE BCAA IPH AVN COMPLEX

2 / EFFICACY ASSESSMENT
OF THE SPORTS NUTRITION PRODUCT
BCAA 2:1:1 + IPH AVN PEPTIDE
COMPLEX IN THE TRAINING PROCESS
OF PROFESSIONAL AND RECREATIONAL
ATHLETES

BCAA IPH AVN AMINO ACID PEPTIDE COMPLEX

Objective of the study

One of the factors that influence athletes' high performance is the intensity of blood supply to actively working muscles.

In this regard, it is necessary to take into account the reactivity of the cardiovascular system, which plays an important role in the processes of adaptation of the body to high physical loads. Indeed, high intensity physical exercises increase the blood flow in muscles by 10–30 times compared to the resting state and amounts to 80% of the minute blood volume. And here lies the problem of increasing the functional activity of the vascular system through intensification of regional blood circulation in order to optimize the work of the heart muscle.

Objective of the study

The aim of this study is to evaluate efficacy of using the BCAA 2:1:1 + IPH AVN Peptide complex, which includes the IPH AVN peptide on the training process of professional and recreational athletes.

To achieve the objective, the following tasks were set

1. To study the effect of the sports nutrition product BCAA 2:1:1 + IPH AVN Peptide complex under a course of treatment (10g three times a day for 30 days) on the regional circulation indices of professional skiers.
2. To evaluate the feasibility of using the sports nutrition supplement with the IPH AVN peptide on the skiers' training process.
3. To evaluate the effect of the product with the IPH AVN peptide under a course of treatment (10g 2 times a day in the morning and evening for 30 days) on the indices of regional circulation of recreational athletes.
4. To evaluate the viability of using the product in the training process of recreational athletes.

Characteristics of participants

The first group — professional athletes

The first group consists of 12 skiers, men (Candidates for Master of Sports, Master of Sports) aged between 18-32. They were divided into 2 subgroups of 6 people — the main and the control.

1 (MAIN) SUBGROUP

6 people — received BCAA 2:1:1 + IPH AVN Peptide complex at a concentration of 10g 3 times a day for 30 days.

2 (CONTROL) SUBGROUP

6 people — received BCAA 2:1:1 without peptides.

Athletes of both groups were in good shape and enjoyed the same conditions: food, medical control, life conditions and the training process.

The second group — recreational athletes

25 recreational athletes (working out 3 times a week for 1.5 hours in a fitness club under supervision of a coach). Participants are men aged 18 to 35 years. They were also divided into 2 subgroups: 15 people — main and 10 people — control.

1 (MAIN) SUBGROUP

15 people — received BCAA 2:1:1 + IPH AVN Peptide complex at a concentration of 10g 2 times a day for 30 days.

2 (CONTROL) SUBGROUP

10 people — BCAA without peptides.

During the study, both subgroups were under medical supervision.

The control group

10 practically healthy men at the age of 18-35 years old, not doing sports and not having intensive physical exercises.

Research methods

Regional blood flow was measured on parallel sections of the right and left thighs of each participant of the experiment at the same time of day (morning 9-11 a.m.) twice during the study period: before the start of the consumption of the sports nutrition products (initial data) and at the end of a 30-day course of treatment.

The indices on the thighs of the same tested subject did not have significant differences, so the tables give average data for each parameter.

Statistical processing of data

Mathematical calculations were carried out in accordance with standard methods used in the processing of medical and biological research results, using the parametric method — Student's t-test, as well as using Spearman's correlation analysis and the factor analysis in Statistica 5.5.



Results of the study

Efficacy Assessment of the Sports Nutrition Supplement BCAA 2:1:1 + IPH AVN Peptide Complex

The results of the study of regional blood flow indices of the tested subjects are represented in Table 1.

Professional sportsmen showed a significantly lower heart rate.

The subjects of the control group not practicing sports had an average heart rate of $67,28 \pm 4,26$ bpm while sportsmen using BCAA and BCAA with peptides had an average heart rate of $57,11 \pm 4,43$ bpm and $53,57 \pm 4,11$ bpm respectively which is significantly lower than the control group by 1,18 and 1,26 times ($p < 0,05$).

Lower heart rate of professional athletes points to a higher rate of functional activity of the heart muscle. In this situation the needs of the myocardium in oxygen decrease, and the diastolic time increases by 1,32 and 1,47 times respectively with sportsmen using BCAA with and without peptides in comparison with the index of the control group.

The heart rate of sportsmen using BCAA + peptide is significantly lower than that of sportsmen using BCAA without peptides which is by 1,1 times ($p < 0,05$).

The diastolic time of sportsmen using BCAA with peptides is greater than the index of sportsmen having BCAA without peptides which is as well by 1,1 times.

The duration of systole in all the groups was not significantly different from the control group.

Such a parameter as the basic resistance characterizing blood filling of vessels in the thigh area was 1.2 times higher among athletes taking BCAA and BCAA + peptide, and 1.26 times higher than in the control group ($p < 0.05$).

Moreover the results of the athletes who took BCAA + peptide were 4% better than in the group taking BCAA without peptides.

Indicators of regional blood circulation of athletes indicate a more intense arterial blood flow than among those individuals having no physical exercise.

Moreover athletes taking BCAA + peptide showed significantly higher indices of the intensity of the arterial blood flow than those athletes taking BCAA without peptides.

Results of the study

Table 1

Effect of the sports nutrition products on the indices of arterial blood flow intensity in the investigated thigh area among the tested subjects

INDEX	CONTROL GROUP n = 10	PROFESSIONAL ATHLETES n = 12		RECREATIONAL ATHLETES n = 25	
		BCAA n = 6	BCAA + IPH AVN n = 6	BCAA n = 10	BCAA + IPH AVN n = 15
Heart Rate, bpm	67,28 ± 4,26	57,11 ± 4,43 ¹	53,57 ± 4,11 ^{1↑}	61,36 ± 5,19	59,35 ± 5,12 ¹
Duration of Systole, sec	0,285 ± 0,056	0,277 ± 0,037	0,272 ± 0,041	0,280 ± 0,057	0,276 ± 0,061
Duration of Diastole, sec	0,589 ± 0,112	0,781 ± 0,096 ¹	0,867 ± 0,102 ¹	0,694 ± 0,123 ¹	0,738 ± 0,109 ¹
Basic Resistance, ohm	132,41 ± 18,16	160,37 ± 21,51 ¹	167,39 ± 20,34 ¹	148,37 ± 22,71 ¹	156,22 ± 19,87 ¹
Rheographic Index, standard units	0,267 ± 0,034	0,663 ± 0,048 ¹	0,796 ± 0,039 ^{1↑}	0,489 ± 0,068 ¹	0,588 ± 0,073 ^{1↑}
Range of Arterial Component, ohm	0,032 ± 0,007	0,071 ± 0,008 ¹	0,085 ± 0,006 ^{1↑}	0,059 ± 0,009 ¹	0,068 ± 0,011 ^{1↑}
Maximum Range of Differential Rheogram, ohm/sec	0,265 ± 0,039	0,693 ± 0,078 ¹	0,758 ± 0,081 ¹	0,431 ± 0,097 ¹	0,594 ± 0,105 ^{1↑}
Amplitude-Frequency index, standard unit	0,295 ± 0,058	0,634 ± 0,076 ¹	0,745 ± 0,089 ^{1↑}	0,416 ± 0,082 ¹	0,542 ± 0,065 ^{1↑}
Relative Volume Pulse, permille	0,228 ± 0,053	0,412 ± 0,067 ¹	0,467 ± 0,087 ^{1↑}	0,356 ± 0,067 ¹	0,410 ± 0,061 ¹
Regional Blood Pulse Volume per minute, ml/min/cm ³	2,375 ± 0,541	4,321 ± 0,776 ¹	4,691 ± 0,472 ¹	3,785 ± 0,732	4,219 ± 0,873 ¹
Rheographic Index, %	0,198 ± 0,032	0,409 ± 0,045 ¹	0,488 ± 0,056 ^{1↑}	0,317 ± 0,073 ¹	0,403 ± 0,093 ^{1↑}
Relative Rheographic Index, standard unit	12,78 ± 2,65	23,51 ± 2,76 ¹	27,45 ± 3,05 ^{1↑}	19,14 ± 3,06 ¹	23,41 ± 3,22 ¹

Table 2

Effect of the sports nutrition products on the indices of arterial tonicity in the investigated thigh area among the tested subjects

INDEX	CONTROL GROUP n = 10	PROFESSIONAL ATHLETES n = 12		RECREATIONAL ATHLETES n = 25	
		BCAA n = 6	BCAA + IPH AVN n = 6	BCAA n = 10	BCAA + IPH AVN n = 15
Ratio b/w quick and slow blood filling	0,678 ± 0,137	0,611 ± 0,122 ¹	0,598 ± 0,094 ^{1↑}	0,651 ± 0,128 ¹	0,625 ± 0,108 ¹
Elastic Modulus, %	20,65 ± 2,76	17,78 ± 2,34 ¹	16,61 ± 1,98 ^{1↑}	18,87 ± 2,67	18,07 ± 3,13 ^{1↑}
Angle of Height, degree	175,46 ± 1,63	163,32 ± 1,52 ¹	155,67 ± 2,52 ^{1↑}	168,54 ± 3,23	160,73 ± 3,17 ^{1↑}

All these data indicate a decrease in the tonicity of small arteries among athletes taking BCAA with or without peptides, but in the first case, the tonicity decreased significantly more.

¹ — Reliable in comparison with the indicator in the control group;
[↑] — Reliable as compared to the index in the group taking BCAA without peptides.

Results of the study

Similar trends are observed with recreational athletes. The ratio of fast and slow blood filling did not differ significantly in the two groups, but there was a tendency to decrease when taking BCAA with the peptide compared to the group taking BCAA without peptides.

Table 3 describes the situation of the tonicity of venous vessels.

Table 3

Effect of the sports nutrition products on the indices of the venous tonicity on the investigated thigh area

INDEX	CONTROL GROUP n = 10	PROFESSIONAL ATHLETES n = 12		RECREATIONAL ATHLETES n = 25	
		BCAA n = 6	BCAA + IPH AVN n = 6	BCAA n = 10	BCAA + IPH AVN n = 15
Diastolic Index, %	56,12 ± 4,64	48,18 ± 5,11 ¹	44,23 ± 4,61 ^{1↑}	51,27 ± 5,18	49,48 ± 6,11 ^{1↑}
Simonson's Index, %	41,16 ± 9,34	32,63 ± 10,19 ¹	27,61 ± 10,12 ^{1↑}	38,17 ± 9,84	34,72 ± 10,32 ^{1↑}
Duration of Catacrotism, sec	0,691 ± 0,122	0,877 ± 0,137 ¹	0,925 ± 0,106 ^{1↑}	0,747 ± 0,133 ¹	0,811 ± 0,131 ¹
Ratio inflow/outflow, standard unit	0,286 ± 0,051	0,231 ± 0,043 ¹	0,207 ± 0,034 ^{1↑}	0,261 ± 0,056 ¹	0,239 ± 0,053 ¹
Ratio of the amplitude of arterial and venous components, %	64,11 ± 8,45	66,32 ± 10,18	65,76 ± 7,98	67,54 ± 11,34	63,61 ± 10,76

¹ — Reliable in comparison with the indicator in the control group;
[↑] — Reliable as compared to the index in the group taking BCAA without peptides.

Thus, the diastolic index of professional athletes taking BCAA and BCAA + peptide is lower by 14.2% and 21.2% compared to the control group.

The ratio of the amplitudes of the arterial and venous components did not differ significantly in all groups of participants. But the time of venous outflow in athletes was significantly greater than in the control group.

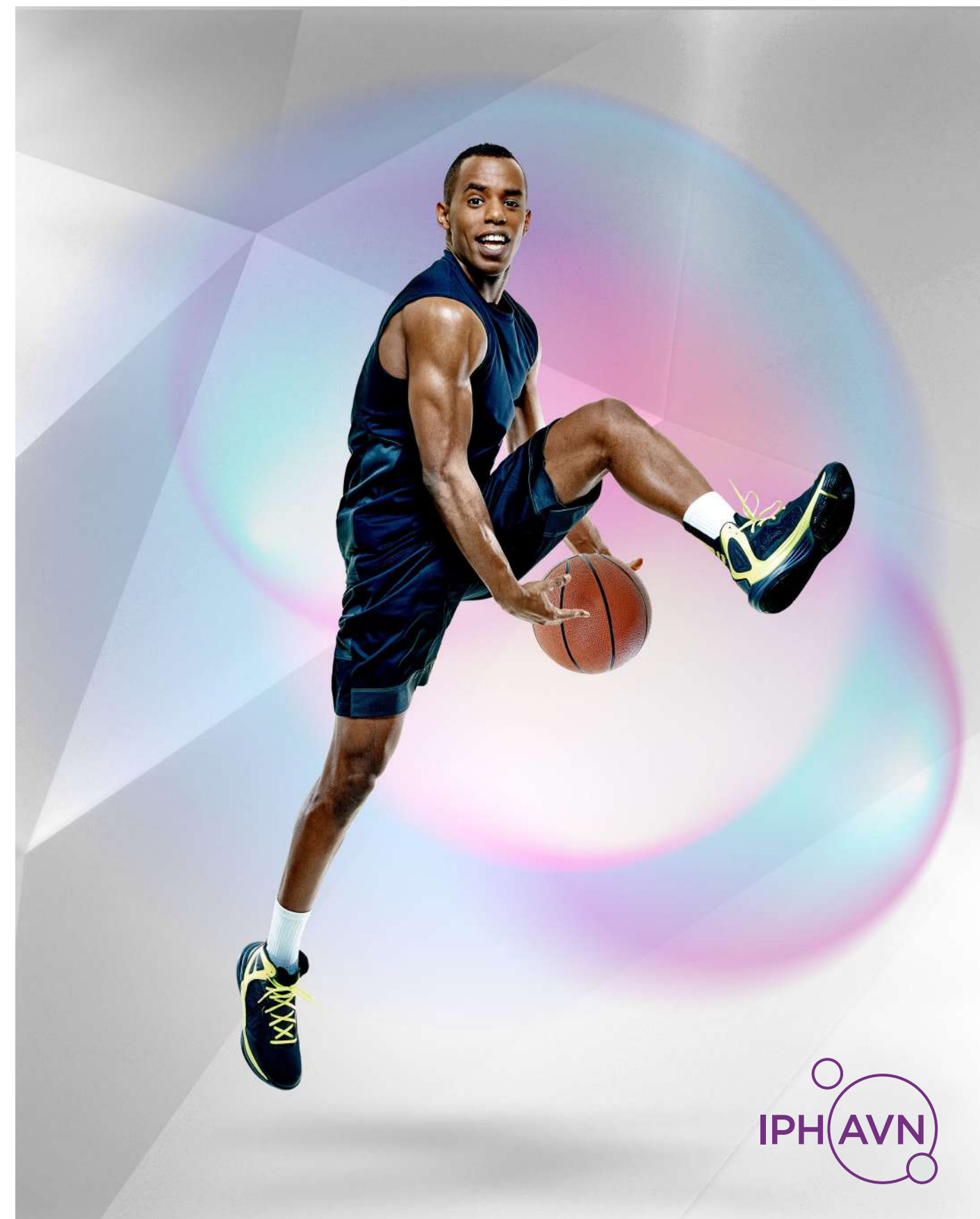
The general venous tonicity of the recreational athletes from the study was significantly higher than in the control group.

Simonson's index was smaller in the groups of athletes taking BCAA and BCAA with the peptide than the indicator in the control group. This indicates that people doing sports and taking BCAA enjoy the best venous outflow.

BCAA IPH AVN AMINO ACID PEPTIDE COMPLEX

Conclusions

1. Analysis of the regional blood circulation studies of the tested subjects from several groups by means of rheovasography showed that athletes' indices of regional hemodynamics are significantly better than those of the control group individuals.
2. The same conclusion can be drawn from the analysis of vascular tone indices: the integral index of arterial tone in those taking BCAA with the peptide was significantly lower than in those who received BCAA without the peptide. But the values in both groups were lower compared to the control.
3. The indices of the venous tonicity indicate significant improvement in the venous outflow among participants taking BCAA + peptide, compared to the parameters of those taking BCAA without peptides.
4. Thus, in order to improve functional activity of the circulatory system, indicators of regional hemodynamics, microcirculation of blood at the level of various blood vessels, it is recommended for professional and recreational athletes to use the sports nutrition supplement BCAA 2:1:1 + IPH AVN Peptide during the training process.



BCAA IPH AVN AMINO ACID PEPTIDE COMPLEX

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