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# Influence of an Amino Acid Composition enhanced with Cold Plasma Radiation on Psychological Stress: A Blood Test, Gas Discharge Visualisation and Biofeedback Approach

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*Abstract* – This study aimed to demonstrate the effect of enhanced amino acid compositions with cold plasma on human psychological stress by using blood tests, biofeedback, and gas discharge visualisation (GDV) techniques for stress measurements. An open, randomised, placebo-controlled trial for 30 days was conducted. 70 healthy people aged 35-65, men and women, were measured initially, randomly divided into three groups (experimental, control, and placebo), and measured 30 days later for changes in stress levels. Twenty people used amino acid composition; 30 used the same amino acid composition processed with cold plasma radiation, while 20 used a placebo. The ethics committee of the Federal State Budget Institution "Saint-Petersburg Scientific-Research Institute for Physical Culture," St. Petersburg, Russia, approved the study protocol. All participants signed an informed consent form, where a written and oral explanation of the research protocol was provided. Blood, biofeedback, and GDV test results were presented. Amino acids processed by the radiation of a cold plasma – enhanced with Igniton particles - had the most significant effect on stress levels. The results suggested that enhanced amino acid compositions significantly affected human stress levels during the longitude period. Stress reduction in humans can significantly influence disease prevention and health maintenance, ultimately extending human life expectancy.

Keywords: Emotional factors, Environmental factors, First-year university students, Social integration

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#### I. INTRODUCTION

WIDELY recognised that amino acids (AAs) serve as cell signaling molecules and play a crucial role in regulating gene expression and the protein phosphorylation cascade. Moreover, AAs have a vital role as a precursor in synthesising hormones and low-molecular-weight nitrogenous compounds with significant biological significance (Du et al. 2018). Nevertheless, heightened concentrations of AAs and their derivatives have been identified as causative elements in the development of neurological diseases, oxidative stress, and cardiovascular disease. Therefore, achieving an ideal equilibrium between AAs intake in the diet and its circulation is of utmost importance for maintaining overall body homeostasis.

An increasing corpus of scholarly works has contributed to the emergence of a novel concept known as functional AAs. This idea refers to AAs as crucial in regulating essential metabolic pathways and enhancing overall well-being (Wu, 2013). AAs have potential medicinal uses in the treatment of stress disorders and anxiety (Hinz et al., 2013). For example, the gamma-amino acid butyric acid (GABA) neurochemical system has been strongly implicated in their pathogenesis and treatment by numerous preclinical and clinical studies for a few anxiety conditions (Goddard, 2016). Another example is the green tea amino acid L-theanine (L-THE), which is associated with several health benefits, including reduction of stress and anxiety-like symptoms (Williams et al., 2020; Ogawa et al., 2018).

Many studies support the idea that stress and anxiety have a significant detrimental effect on people's bodies and, consequently, their health. Lifestyle stressors have been related to adverse health consequences like cancer, gastrointestinal distress, and coronary heart disease. These stressors can have a detrimental effect on human survival and, eventually, shorten human life expectancy. Literature has been published in the previous 50 years that supports the idea that the individual's psychological state affects the immune system. Better stress management can significantly influence disease prevention and health maintenance, ultimately extending human life expectancy (Vitetta et., 2005)

The proposed AA composition for the study has the following content that aims to reduce stress levels and promote longevity.

- Nicotinamide Ribose
- Resveratrol
- Reduced Glutathione
- Berberine
- L-5MTHF (Calcium salt)
- PQQ (B14)
- Cycloastragenol
- Bioperine

Plasma is a state of matter characterised by ions and electrons. The plasma in question is also known as a cold plasma or non-equilibrium plasma, and the temperature differences between its constituents characterise it. Neutral atoms are at an ambient temperature, while electrons are at a higher temperature. Nevertheless, the density of the electrons in the plasma is significantly lower than that of the neutral elements. In the laboratory, frigid plasmas are typically generated by applying electrical energy to various inert gases. There are two particularly intriguing aspects of cold plasma. Initially, cold plasma is a source of high-temperature electrons. Secondly, cold plasma generates numerous reactive species when interacting with a controlled environment. These reactive species can be employed in various chemical reactions across various scientific disciplines. Cold plasma is a gaseous state characterised by partial ionisation. The composition of the substance consists of a combination of neutral atoms, atomic ions, electrons, molecular ions, and molecules in both excited and ground states. The quantity of particles present in plasma is contingent upon the gas pressure under which it was generated. The positive and negative charges exhibit a state of equilibrium, resulting in a significant proportion of these charges being electrically neutral (Niemira 2012).

In this paper, data that demonstrate the advantage of using an enhanced cold plasma AA composition to treat stress disorders for the promotion of longevity is presented and compared with the same AA composition but non-enhanced and a placebo. The proposed study is for an AA composition with cold plasma that can be used to treat stress disorders and promote longevity. This study proposes blood tests, gas discharge visualisation, and biofeedback to measure stress levels in individuals included.

### **II. LITERATURE REVIEW**

Cold plasma

Cold plasma in food preparation has recently become popular (Gao et al., 2023). The purpose of its application is to mitigate the occurrence of microbial contaminations (Schnabel et., 2015). There are several notable benefits associated with cold plasma, including the extension of product shelf-life (Pan et al., 2019), enhancement of food drying performance in food production (Du et al., 2022), and the potential preservation or improvement of food's organoleptic and biomolecular qualities following processing (Lopez et al., 2019).

An emerging field adds the capabilities of cold plasma to dentistry and medicine. Cold plasma treats chronic wounds (Abu Rached, 2023) and Surface Treatments for Medicine (Tabares et al., 2021). It has received considerable interest due to its inherent benefits, including free radicals for the inactivation of microbes, eco-friendliness, cheap operational cost, simplicity of operation, and portability of devices (Deepak, 2022). There are hypotheses that plasma radiation can generate a particular type of non-electromagnetic field (or quasi-particles) known as ignitions, related to tachyon or scalar field (Park & Park, 1997; Chiao et al., 1996), and this may be the reason for significant effects of the enhanced AA compositions.

Valverde et al. (2023a; 2023b) presented results of the study of the influence of enhanced amino acid (AA) compositions with cold plasma on human cognitive functions, including people after COVID-19. It was shown that using enhanced AA compositions during the longitude period significantly affected cognitive functions, particularly memory, speed of reactions, and attention, which allowed overcoming the Covid inflammation's negative effects.

Stress measurements with blood tests, GDV, and biofeedback

Several types of blood tests were used in this study. Stress can cause hypertension through repeated blood pressure elevations and by stimulating the nervous system to produce large amounts of vasoconstricting hormones that increase blood pressure. Hypertension can be measured with a systolic blood pressure test (greater than 139 mm Hg) and a diastolic blood pressure test (greater than 89 mm Hg). This is because stress can lead to repeated blood pressure elevations, which eventually may lead to hypertension (Kulkarni et al., 1998).

Systolic blood pressure (SBP) is the higher number in a blood pressure reading and indicates the pressure of blood against artery walls when the heart beats. Stress can cause a temporary increase in blood pressure due to the body releasing hormones that make the heartbeat faster and narrow blood vessels. Kurl et Al. (2001) conducted a study that used SBP to show a relationship between SBP during exercise tests and stroke by measuring the stress produced by exercise.

The C-reactive protein in blood tests can also measure stress levels. Kennedy and Niedzwiedz (2022) conducted a study to assess the relationship between different anxiety/stress-related disorders and inflammation measured by C-reactive protein in blood tests. In this study, four logistic regression models were calculated in which the associations between anxiety/stress disorders and C-reactive protein (CRP) >3 mg/L were established.

GGT (gamma-glutamyl transferase) is an enzyme found in the liver, kidneys, pancreas, heart, and brain. A GGT blood test measures the level of GGT in the blood and can help diagnose liver or bile duct diseases, screen for alcohol use, and differentiate between liver or bile duct disorders and bone disease. Critical increases in stress at work can be related to increases in GGT (Tomei et al., 2016). The test for Interleukin-6 can also be useful in stress measurements, Interleukin-6 is significantly positively associated with one's present experience of depressive and anxiety symptoms, according to Lee (2020),

Kostyuk et al. (2011) introduce gas discharge visualisation (GDV) as a nonintrusive technique that captures a person's physiological and psycho-emotional status, including stress. This is through the electrophotonic emissions of fingertips placed on the surface of an impulse analyser. The GDV technique (Korotkov, 2004) places an object in a highly intensive electromagnetic field (EMF). The object is separated from the electrode by a dielectric – a substance that does not conduct electricity but permits an electromagnetic field. With this arrangement, if we apply a voltage between the object and the electrode, the current does not flow, but a potential difference builds up until the breakdown voltage is reached.

This is the point at which electrons around the object begin to move, and a current begins to flow along the surface of the dielectric. As they move, the electrons collide with heavier gas molecules, wrenching out electrons and emitting quanta of light (photons). Each collision results in two electrons, producing a branching tree-like light pattern. When an alternating current is used, avalanches of ionisation moving away or towards the electrode, core are overlaid upon each other. The GDV uses a camera to photograph the emitted photons and a computer programme to analyse the images to determine energy levels of different electromagnetic fields that carry quantum information that can be interpreted for different applications, including stress measurements. Then, data is collected using a GDV device to identify the functional psycho-emotional state of a person using fingertips (including stress). The analysis of natural electro-photonic emission is based on the intensity, fractality, and area of the captured images (Korotkov, 2004).

Kostyuket et al. (2010) reported a study using computational bioelectrography based on the gas discharge visualisation (GDV) technique to investigate an individual's physiological and psycho-emotional functional states. The study evaluated anxiety while learning English as a Second Language. Rastogi et al. (2021) present a model to measure the different biophysical factors and relate them to different human illnesses and psychological conditions such as stress. Valverde et al. (2023) propose using GDV to measure stress levels in altered states of consciousness.

Biofeedback has been used for stress measurements by using different technologies (Valverde, 2023). Biofeedback is the process of acquiring the ability to influence involuntary bodily processes to receive physiological data from an electronic device that perpetually monitors specific physiological parameters. It is a technique for evaluating individuals' responses to physical, emotional, mental, and spiritual stressors. Physical discomfort and illness are more likely to affect individuals who are stressed. The body responds to new information about its status by making healthful adjustments to alleviate stress and tension. Electrical measurements obtained from the frontal cortex of the body are the foundation of biofeedback. The main objective of biofeedback is to teach the patient to consciously alter their internal reactions to alter the electrical results measured with biofeedback when the patient faces stress and tension.

Blood flow, brain electrical activity, muscle activity, skin temperature, electro-dermal activity (sweat gland activity), respiration, heart rate variability, and blood pressure are all measured using biofeedback instruments. Heart rate variability (HRV), which detects the physiology of emotions, is an important tool that provides a window to the activity between the heart and the brain. This gives us an instrument to measure emotional impact on people. HRV variables are used to assess beat-to-beat changes in heart rate associated with rhythms generated by different physiological mechanisms. An appropriate level of physiological variability in regulatory systems reflects the flexibility and ability of an organism to consistently adapt to stress and challenges (Valverde, 2023) (Valverde, 2016). An example is a stress Management System developed by Al Osman et al. (2014) that is intended for users interested in detecting potential stress manifestations using HRV for stress measurements.

## Proposed amino acids for the composition

The proposed AA composition (IgniLongevity) is a multi-component orthomolecular preparation consisting of 8 food supplement metabolites of the body or substances of natural origin. The unique composition of the IgniLongevity was selected to improve the quality and duration of life by regulating and enhancing metabolic functions that slow down the development of age-related diseases, protect DNA from mutations, and provide anti-stress properties. The bioperine contained in IgniLongevity increases the bioavailability of other preparation components, contributing to their more effective use by the body.

## Nicotinamide riboside

Nicotinamide riboside is a form of nicotinic acid (vitamin B3), a product of yeast enzymatic activity that enters the body with food. It is a precursor of nicotinamide adenine dinucleotide (NAD+), a coenzyme that regulates energy production in the body (Chi & Sauve, 2013). Nicotinamide riboside has a unique metabolic pathway to NAD+. A deficiency of NAD+ in the body triggers the aging processes and reduces insulin sensitivity, immunity, cardiovascular health, liver health, and other metabolic functions as the body ages. NAD+ production decreases, leading to obesity. Nicotinamide riboside supplementation has immunomodulatory effects and reduces chronic inflammation characteristic of aging, promoting longevity through improved metabolism (Mehmel, Jovanović & Spitz, 2020). The administration of nicotinamide riboside to patients suffering from coronavirus infection has been shown to prevent virus transmission by inhibiting cytokine production (Gharote, 2021). Nicotinamide riboside is currently being studied in many clinical trials, including immunity, cognitive function, metabolic disorders, stress disorders, and ageing. According to Peng et al. (2024), this AA can be a strong anti-stress supplement and, therefore, included in the proposed AA composition for its anti-stress and longevity promotion properties.

### Resveratrol

Resveratrol is a polyphenol - a secondary metabolite produced by plants to protect them from abiotic factors (drought. extreme temperatures. flooding. heavy metals, pH radiatio soil salinity) and biotic factors such as pathogen attack. Polyphenols are natural antistress factors developed by evolution to help plants adapt to changing environmental conditions. Resveratrol is found in the skins of grapes, other fruits, cocoa nuts, and red wine. A natural antioxidant for humans, resveratrol reduces the production of reactive oxygen and nitrogen species that damage cells. There are many publications on resveratrol's including cardioprotective, neuroprotective, clinical effects. phytoestrogenic anti-aging, anti-inflammatory, hepatoprotective, and metabolic effects. Resveratrol supports the elasticity of the skin, heart blood vessels, immune system, and brain function, normalises blood sugar levels, neutralises inflammatory processes, and has a positive effect on male and female reproductive systems (Fabjanowicz, ka-Wasylka & Namieśnik, 2018). It has been shown that resveratrol can be

used as a safe drug to inhibit Wnt signaling. one of the intracellular signaling pathways that regulate cancer development (Chen et al., 2012). Anti-stress properties of resveratrol were also found by Vetvicka and Vetvickova (2014), who evaluated the stress-reducing effects of the resveratrol- $\beta$ -glucan-vitamin C combination.

Glutathione reductase

Over the years, several theories have been formulated to explain the molecular mechanisms of age-related diseases. Much attention has been given to the free radical theory of aging (Harman 1992), which states that aging and related degenerative diseases are caused by the accumulation of damage caused by reactive oxygen species (ROS). Sixty-seven years after this theory was put forward. It has become clear that free radicals are involved in the pathophysiology of aging in complex ways. It is a certain level of reactive oxygen species generation, especially at the mitochondrial level with moderate oxidative stress, can induce endogenous defense mechanisms with increased stress tolerance resilience and longevity, a process known as "mitohormesis" as an extension of the broader term "hormesis" (Lapenna, 2023). Glutathione reductase is a key molecule resistant to oxidative stress, reducing the production of reactive oxygen species and maintaining the membrane stability of all cells in the body.

Berberin

Berberine is a natural alkaloid. It is found in various parts of many plants belonging to different families and is one of the most common substances in plants. Berberine has been used as a medicine in China since ancient times. It was mentioned in Shen-nun as early as 3000 BC. It has a choleretic and antispasmodic action that reduces the tone of the gallbladder musculature, reduces the amplitude of its contractions, promotes bile secretion, and thus cleanses the liver and the whole organism. Berberine has many valuable pharmacological properties that have found their application in treating and preventing various diseases, including cancer, heart disease, and diabetes mellitus (Gao et al., 2020; Patel, 2021; Malhotra et al., 2021). Berberine has neuroprotective properties, protecting the body against aging atherosclerosis and age-related loss of learning ability (Xu et al., 2017; Wang et al., 2019).

### L-5MTHF (Calcium salt)

L-5-MTHF-Ca is intended for use in dry crystalline or microencapsulated form as an alternative to folic acid (sometimes known as Vitamin B9), in dietary supplements, particularly special dietary uses, and other foods for fortification. L-5-MTHF-Ca is the calcium salt of naturally occurring L-5-methyltetrahydrofolate acid (L-5-MTHF), the latter acting in the body as an enzyme cofactor for the transport of C1 in methylation reactions in the biosynthesis of pyrimidines, purines, serine, and glycine.

### Pyrroloquinoline quinone (PQQ)

PQQ, known as methoxatin and vitamin B14, is a redox cofactor and antioxidant. It is found in foods such as kiwi and dark chocolate. The antioxidant activity of PQQ is 100 times greater than that of vitamin C and all known antioxidants. PQQ can boost immunity, increase physical and mental performance, improve musculoskeletal health, and protect the central nervous system from stress. PQQ increases the body's energy reserves and improves overall quality of life at any age. Some of the most critical effects of PQQ include strengthening blood vessel walls, maintaining heart muscle, improving and restoring reproductive function in men and women, improving memory and attention, lowering cholesterol, hepatoprotective effects, and eliminating insomnia, anxiety, chronic fatigue, and lethargy, and other stressrelated disorders (Jonscher et al., 2021). PQQ's main effect is activating mineralocorticoid receptor antagonists, thereby influencing the energy potential of cells. This helps preserve youth by slowing the ageing process and accelerating fat burning in the body when taking pyrroloquinoline. Yang et al. (2021) emphasise the anti-stress property of PQQ for longevity purposes.

Cycloastragenol

This is a natural tetracyclic triterpenoid extracted from the root of

Astragalus. This plant has been used in traditional Chinese medicine for over 2000 years. Cycloastragenol has antioxidants, antibacterial, antiinflammatory wound healing, and immune-stimulating properties. At the same time, it can also reduce hypersensitivity to infection. The antiaging and anti-stress properties of cycloastragenol are particularly valued in the proposed blend. The anti-aging effect of the compound is associated with an increase in the activity of the enzyme telomerase. It is currently the most important compound known to induce telomerase in humans, making it a promising addition to further developing the field of anti-aging medicine. By measuring telomere length, it is possible to assess the ageing process of cells and determine whether their size corresponds to biological age (Ye et al., 2023). This test is now available in most major laboratories Telomerase activation helps to stabilise chromosomes maintain their integrity, and prevent degradation, fusion, and abnormal recombination of DNA strands. In the review by Yu et al. (2018), Cycloastragenol is an exciting new candidate for the treatment of age-associated diseases" shows that this supplement improves longevity in humans and animals.

Bioperine

Bioperine improves the absorption and assimilation of minerals. B vitamins, plant extracts, and other substances with low bioavailability can improve the body's ability to use many other substances efficiently. In addition, bioperine has numerous potential health benefits: it prevents inflammation and oxidative stress, has sedative anticonvulsant and antidepressant properties, improves cognitive function and mood, and reduces allergies, cholesterol, and blood pressure. The antioxidant effect of bioperine is due to its direct action as a scavenger of hydroxyl and superoxide radicals. It has been shown to increase dopamine and serotonin levels in the brain, improving mood and cognitive function. Bioperine has potent antidepressant properties mediated partly by inhibiting monoamine oxidase activity (Lee et al., 2005). Bioperine improves muscle metabolism by increasing ATPase activity. This may increase muscle energy intake and aid in weight loss. (Nogara et al., 2016). Bioperine can produce significant antianxiety-like activity and be used as a practical anti-stress and anxiety component (Gilhotra & Dhingra, 2014).

## III. MATERIALS AND METHODS

#### Participants

Seventy adults of different sexes, ages, and professions, aged 50 + / - 14 years, participated in the study. Each subject received information about the study before the study and signed the Informed Consensus Form. Information obtained during the investigation that identifies the identity of the subjects was kept secret and may be disclosed only within limits established by the law. The protocol was approved by the Ethics Committee of the North-Western Medical University, St. Petersburg, Russia. The study included adults meeting the following criteria:

- an absence of severe chronic medical conditions,

- good mental health,

- the ability to comply with the procedures set out in the inquiry protocol.

There was no early abandonment of the subjects from the experiment.

Research design

Participants were randomly selected into three groups:

Group 1: 30 people consumed daily amino acid composition processed with plasma radiation (enhanced with Igniton particles) for 30 days.

Group 2: 20 people for 30 days consumed the same amino acid composition without processing.

Group 3: 20 people for 30 days consumed daily placebo.

In the literature review section, several indicators of physiological stress were discussed that significantly reduce the quality and duration of life and contribute to the development of age-related diseases. These are interleukin-6 (IL-6), heart rate variability, GDV measurements, GGT (gamma-glutamyl transpeptidase) as an indicator of endogenous intoxication, systolic and diastolic blood pressure and levels of the CRP C-reactive protein. Cardiac activity (HR. BP. heart rate variability) is quite significant in screening physiological stress (Sumińska, 2022; Georgiou et al., 2018). The GDV device used to measure stress using the stress index is Bio-well (Korokov & Yanovskaja, 2024).

#### IV. HYPOTHESES OF THE STUDY

Two hypotheses are considered for this study. The complete set of hypotheses is below:

• H1: No difference in stress levels exists in people who consumed amino acid composition processed with plasma radiation.

• H2: There is no difference in stress levels in people who consumed processed amino acid composition.

#### V. RESULTS

The initial test results and after every month are presented in Table 1. Table 1 shows the stress indicators before and after one month of the intake of the AA composition for the different groups in the study. The table also contains the p values for the t-student test for a difference in the mean values over one month for the different groups in the study. *Table 1: Results of the AA composition intake for 30 days in different groups* 

| Indicator   | Initial   | I<br>mont<br>h<br>later | Differ. | Initial   | I<br>mont<br>h<br>later    | Differ. | Initial                 | I month<br>later | Differ. |
|---|---|-------------------------|---------|---|----------------------------|---------|-------------------------|------------------|---------|
|   | Experimental group 1 - AA<br>composition enhanced with<br>Cold plasma |                         |         | Experimental group 2 - Non<br>- enhanced AA composition |                            |         | Control group - Placebo |                  |         |
| Systolic<br>blood<br>pressure<br>(mmHg)           | 127.7<br>±12.8  | 118.0<br>±10.4<br>***   | 0.0003  | 123.5<br>±9.2   | 121.5<br>±10.0             | p>0.05  | 120.5<br>±10.9          | 124.3<br>±11.6   | p>0.05  |
| Diastolic<br>blood<br>pressure<br>(mm.Hg)         | 79.6<br>±9.7  | 72.1<br>±9.4<br>***     | 0.0004  | 77.5<br>±9.0  | 77.5<br>±10.6              | p>0.05  | 73.0<br>±9.5            | 71.8<br>±8.8     | p>0.05  |
| GGT<br>(U/L)/<br>GGT                              | 25.4<br>±28.3   | 22.2<br>±19.8<br>*      | 0.04    | 21.3<br>±10.9   | 25.8<br>±14.1              | p>0.05  | 18.5<br>±10.8           | 18.1<br>±9.17    | p>0.05  |
| Interleuk<br>in-6<br>(Pg/ml)                      | 1.39<br>±1.56   | 0.88<br>±1.00<br>**     | 0.01    | 0.75<br>±0.38   | 0.77<br>±0.43              | p>0.05  | 1.02<br>±1.37           | 1.19<br>±1.72    | p>0.05  |
| CRP C-<br>reactive<br>protein.<br>(mg/L)          | 2.13<br>±2.05   | 1.54<br>±1.27<br>**     | 0.01    | 1.42<br>±1.55   | 1.48<br>±<br>1.70          | p>0.05  | 3.07<br>±6.21           | 2.47<br>± 3.68*  | 0.02    |
| HRV<br>Autonomic<br>equilibriu<br>m index<br>(SI) | 338.9<br>±224.7   | 328.1<br>±123.<br>4 *** | 0.002   | 423.0<br>±197.1   | 360.4<br>±202.<br>7<br>*** | 0.002   | 0.68<br>±0.36           | 0.80<br>±0.38    | p>0.05  |
| HRV<br>Stress<br>index                            | 210.8.1<br>±140.3   | 206.8<br>±82.7<br>***   | 0.007   | 282.1<br>±156.6   | 236.6<br>±158.<br>5        | p>0.05  | 216.1<br>±134.6         | 199.9<br>±161.1  | p>0.05  |
| Bio-Well<br>Stress<br>index                       | 4.39<br>±2.74   | 3.50<br>±0.52<br>***    | <0.001  | 3.29<br>±0.42   | 3.50<br>±0.65              | p>0.05  | 3.61<br>±0.39           | 3.57<br>±0.62*   | 0.02    |

Significant differences: \* - p<0,05, \*\* - p<0,01, \*\*\* - p<0,001

#### VI. DISCUSSION

From Table 1, a month of taking the AA composition enhanced with cold plasma (2 capsules a day) showed positive, reliable dynamics in most studied parameters. Hemodynamic parameters significantly improved: systolic and diastolic BP decreased. According to HRV and Bio-Well (GDV) data, the stress of adaptation mechanisms decreased. There was a significant decrease in interleukin-6, GGT, and CRP, which reflects the reduction of systemic inflammation and endogenous intoxication. These results support the rejection of the H1 hypothesis as it supports a difference in the means of stress level indicators with a significant level of p<0.01. In the second and control groups, no significant differences were identified in most of the parameters studied, which supports the acceptance of hypothesis H2.

The presented results demonstrate that using the enhanced AA

compositions significantly affects stress levels, while in group 2 and placebo, results practically did not change. This testifies that regular intake of enhanced AA composition positively affected stress levels.

One limitation of this study is its lack of generalisability since it involved only one data set. Many critics question the academic value of the case study method as they argue that the finding or results can not be generalised (Valverde et al., 2011). The limitations of this study include the sample size since we are using convenience sampling, which can compromise the accuracy of the results (Valverde et al., 2011).

Longer periods for the AA-enhanced composition intake would also be beneficial in finding the thresholds for the positive effect for usage recommendation purposes.

## VII. CONCLUSION

When comparing the three groups involved in the study, reliable positive changes were found in the group taking the AA-enhanced composition. After one month of taking the AA enhanced composition, the following significant changes were observed in the group that took this AA: improvement of hemodynamic parameters, adaptive characteristics, and antioxidant status of the organism, reduction of tension in regulatory systems and energy deficiency, systemic inflammation, endogenous intoxication, impact of stress factors on the organism. In the other two groups, most indicators did not change at p<0.01 significant level.

Regular intake of enhanced AA compositions during the longitude period significantly affected stress levels, which may be interpreted as the anti-stress properties of cold plasma in AA compositions. Future research can be conducted to confirm this hypothesis with further studies with longer periods and comparisons with pharmaceutical anxiolytics for effectiveness studies.

## VIII. TRANSPARENCY STATEMENT

We reported how we determined the sample size and the stopping criterion. We reported all experimental conditions and variables. We report all data exclusion criteria and whether these were determined before or during the data analysis. We report all outlier criteria and whether these were determined before or during data analysis.

#### IX. CONFLICTS OF INTEREST

There are no conflicts of interest.

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