

Studying of the Factors of Longevity in Smolyan Municipality, Rhodope Mountains, Bulgaria as Area of Oxidant/Antioxidant Balance

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Abstract

This paper shows the research of factors for health and longevity in Smolyan Municipality, Bulgaria in Rhodope Mountains. The factors are – hereditary, gender, mountain water, body weight, food, movement, psychological status, family relationships. This area is popular with mountain water and yogurt and higher altitude above sea level (over 1000 m altitude above sea level). Yogurt is typical as food in Rhodope Mountains. Natural mountain waters were studied from various Bulgarian water springs from the period of 2010-2018. In 1910 in Bulgaria lived 2067 centenarians from 4 337 513 inhabitants. There were 477 centenarians per million. The most of these people lived in Rhodope mountains. There are the most centenarians in Europe. In the early 1900s, the Bulgarian scientist, Stamen Grigorov, found the specific *Lactobacillus* bacteria responsible for Bulgarian yogurt fermentation. Interested in Grigorov's discoveries, the Nobel prize-winning, Russian scientist, Ilya Mechnikov, noted that more people lived to the age of 100 in Bulgaria than in any of the 36 other countries he studied. He directly linked this to Bulgaria's consumption of yogurt. According to Metchnikoff, aging is promoted by putrefactive microbes in the large bowel. He knew that milk fermented with lactic acid bacteria inhibits the growth of toxic bacteria, because of the low pH (4.4) produced from lactose fermentation. Metchnikoff proposed that fermented milk would "seed" the intestine with lactic acid bacteria, decrease intestinal pH, and suppress growth of toxic bacteria. Metchnikoff named the primary yogurt microorganism *Lactobacillus bulgaricus* after the Bulgarians. In the early 1900s, Mechnikov worked to popularize yogurt as a foodstuff throughout Europe. There was performed research of mountain water from village of Momchilovtsi, Smolyan Municipality. There was performed study of blood serum of cancer patients between 50 and 70 years old were investigated by IR, NES and DNES-methods (Ignatov&Mosin, 2012). As estimation factor was measured the values of the average energy of hydrogen bonds ($\Delta E_{H...O}$) among H_2O molecules, as well as local extremums in DNES- and IR-spectra of various samples of water and human blood serum, detected at -0.1387 eV and wavelength – 8.95 μm . For a group of people in critical condition of life and patients with malignant tumors the greatest values of local extremums in DNES- and IR-spectra are shifted to lower energies relative to the control group. The IR-spectrum of mountain water is most similar to the IR-spectrum of blood serum of healthy group of people with a local extremum at $\lambda = 8,95 \mu m$. The authors study the local extremum at $\lambda = 8,95 \mu m$ of water in Momchilovtsi. This is the indicator for preservation of energy among water molecules in people, who are living in near the mountain sources. The authors show in the report that mountain water and yoghurt are part of secret of longevity. They and also natural food contain whole ballasting microelements – Calcium (Ca), Magnesium (Mg), Potassium (K), Sodium (Na), Manganese (Mn), Zink (Zn). The author shows that in Smolyan Municipality, Rhodope Mountain there is balance between oxidant and antioxidant activity in the human organism of the people, who are living here. The report shows the proofs.

Keywords: longevity, mountain water, yoghurt, factors of longevity, oxidants/antioxidants

1. Introduction

Yoghurt has probiotic effects in digestive system on the base of *Lactobacillus bulgaricus*. *Lactobacillus bulgaricus* is very adaptive (Van de Guchte, M. et al., 2006). Part of the intestinal mucosal barrier function is formed by a common mucosal immune system which provides communication between the different mucosal surfaces of the body. The intestinal colonisation with a balanced microflora is of main importance for the correct development of the immune system. The use of probiotics or prebiotics to correct this imbalance and modulate the immune activity. (Ouweland, Isolauri & Salminen, 2002). The research shows anti inflammatory effects of *Lactobacillus bulgaricus* on 35 hospital patients (mean age 74) taking antibiotics (Hickson et al., 2007). The inflammations are part of aging process. Bulgarian yoghurt influences decreasing inflammations with anti aging effect (Ignatov, Pesheva, 2018). Research of Bulgarian team shows the antimicrobial effects of Bulgarian yogurt. *Lactobacillus bulgaricus* BB18 and *E. faecium* MH3 produce two novel bacteriocins highly similar to the pediocin - like nonantibiotics. The two bacteriocins are potential antimicrobial agents and, in conjunction with their producers, may have use in applications to contribute a positive effect on the balance of intestinal

microflora. Furthermore, bulgaricin BB18 strongly inhibits *Helicobacter pylori*. (Simova, E., Beshkova, D.&Dimitrov, Zh., 2009). There is antibiotic resistances in Bulgarian Yogurt (Sozzi, T&Smiley, M., 1980). Water is the main substance of life.

The human body is composed from 48 to 54% of water for adult people. With aging, the percentage of water in the human body decreases. Hence, the factor of water quality is the essential factor for the research (Pocock *et al.*, 1981; Howard & Hopps, 1986). Water is present in the composition of the physiological fluids in the body and plays an important role as an inner environment in which the vital biochemical processes involving enzymes and nutrients take place. Water is the main factor for metabolic processes and aging (Ignatov, 2012). Earlier studies conducted by us have demonstrated the role of water, its structure, isotopic composition and physico-chemical properties (pH, temperature) in the growth and proliferation of prokaryotes and eukaryotes in water with different isotopic content (Mosin & Ignatov, 2012; Ignatov & Mosin, 2013). These factors and the structure of water are of great importance in biophysical studies. The peculiarities of chemical structure of H₂O molecule create favorable conditions for formation of electrostatic intermolecular van der Waals, dipole-dipole forces and donor-acceptor interactions with transfer of charges between H-atom and O-atoms in H₂O molecules, binding them into water associates (clusters) with the general formula (H₂O)_n where n varies from 3 to 50 units (Saykally, 2005; Ignatov, Mosin, 2013). The structuring clusters with water molecules at $\lambda = 8,95 \mu\text{m}$ with drinking of mountain water has important role for biochemical process. The basic village Momchilovtsi of the research is with 1200 m attitude above sea level. Of this level has more water clusters at $\lambda = 8,95 \mu\text{m}$. There is process of cleaning of water in spring and melt water is part of the life of the areas with long living people and centenarians. When the people are living of the attitude above sea level more than 800-1000 m there is increasing of number of erythrocytes. Hemoglobin (Hb), packed cell volume (PCV), red blood cells (RBCs), mean cell volume (MCV), and mean cell hemoglobin concentration (MCHC) were measured by a single operator in a single laboratory by means of an electronic particle counter in 942 healthy adults (491 females and 451 males) residing at five altitudes: 0, 1000, 1860, 2220, and 2670 meters above sea level. The subjects were carefully screened clinically, and subjects with low transferrin saturation (< 15%) were excluded. In both sexes there was a differential behavior as a function of altitude, of Hb and PCV on the one hand, and number of RBCs on the other. The findings suggest the presence of two sequential mechanisms of adaptation to progressively lower atmosphere oxygen pressure: One operating from sea level to 1860 meters, which leads to a progressively increasing number of relatively microcytic RBC. (Ruiz-Arguelle, G.J. et al., 1980).

Reactive oxygen species (ROS) are highly reactive molecules and can damage cell structures. The shift in the balance between oxidants and antioxidants in favor of oxidants is termed “oxidative stress.” In human organism there are antioxidant systems, which include enzymatic and nonenzymatic antioxidants that are usually effective in blocking harmful effects of ROS. Oxidative stress is result of different diseases and pathological conditions – cancer, atherosclerosis, diabetes, hypertension, ischemia/perfusion, acute respiratory distress syndrome etc. The research shows that long living people and centenarians in Rhodope Mountains are healthy people and this is effect of balance oxidants/antioxidants as result o manner of life and heredity.

2. Material and Methods

2.1. Preparation of melt water

The melt water was obtained from Moscow tap water by the freeze-thaw method in a standard procedure: 1,5 l of Moscow tap water was placed in a glass jar with a lid and placed in the refrigerator freezer at -14 °C for 4–5 hours. Then, the first ice crystals were mechanically removed from the mixture, and the jar again was placed in the freezer additionally for 8–10 hours before $\frac{3}{4}$ of liquid freezes. Thereafter, the liquid brine is decanted and the remaining ice was thawed at room temperature and used for further experiments. The melt water was stored in a glass container in refrigerator. Other experiments were carried out with deuterium depleted water (DDW) with residual deuterium content of 60–100 ppm, purchased from Langway Water Inc. (Moscow, Russia).

2.2. NES and DNES Spectral Analysis

The device for DNES was made from A. Antonov on an optical principle. In this study was used a hermetic camera for evaporation of water drops under stable temperature (+22–24 °C) conditions. The water drops are placed on a water-proof transparent pad, which consists of thin maylar folio and a glass plate. The light is monochromatic with filter for yellow color with wavelength $\lambda = 580 \pm 7 \text{ nm}$. The device measures the angle of evaporation of water drops from $72,3^\circ$ to 0° . The spectrum of hydrogen bonds among H₂O molecules was measured in the range of -0.08– -0.1387 eV or $\lambda = 8.9–13.8 \mu\text{m}$ using a specially designed computer program. The main estimation criterion in these studies was the average energy ($\Delta E_{\text{H...O}}$) of hydrogen O...H-bonds between H₂O molecules in human blood serum.

2.3. Studying the Bulgarian Centenarians from Smolyan Municipality

Interviews have been conducted with 415 Bulgarian centenarians and long living people and their siblings. 2012-

2017. In 2018 in Smolyan Municipality were studied 62 long living people and centenarians and their siblings. The participants in project are 477. Their heredity, body weight, health status, tobacco consumption, physical activity, attitude towards life has been analyzed. With using NES method was performed a spectral analysis of mountain water spring located in Smolyan Municipality (Bulgaria) – Holly source-Momchilovtsi. The composition of water samples was studied in the laboratory of “Eurotest Control” (Bulgaria). Statistics methods were attributed to the National Statistical Institute of Bulgaria.

2.4. Studying the human blood serum

For the period 1965-1975 Argir Hadzhihristev performed blood tests of 54 centenarians in Smolyan District. 1% (v/v) solution of human blood serum was studied with the methods of IR-spectrometry, non-equilibrium (NES) and differential non-equilibrium (DNES) spectral analysis. The specimens were provided by Kalinka Naneva (Municipal Hospital, Teteven, Bulgaria). Two groups of people between the ages of 50 to 70 were tested. The first group (control group) consisted of people in good clinical health. The second group included people in critical health or suffering from malignant diseases (Ignatov, 2012).

2.5. IR-spectroscopy

IR-spectra were registered on Brucker Vertex (“Brucker”, Germany) IR spectrometer (a spectral range: average IR – 370–7800 cm^{-1} ; visible – 2500–8000 cm^{-1} ; the permission – 0,5 cm^{-1} ; accuracy of wave number – 0,1 cm^{-1} on 2000 cm^{-1}).

2.6. Clinical testing

Clinical testing was performed from Argir Hadzhihristev in the period from 1965 to 1975 for 54 centenarians from Smolyan region. The research was included – blood testing, enzymes, diagnostic of whole health status. The author was made the study of whole health status.

2.7. Statistical Processing of Experimental Data

Statistical processing of experimental data was performed using the statistical package STATISTISA 6 using the Student's *t*- criterion (at $p < 0.05$).

3. Results and Discussions

3.1. Comparative Analysis between Longevity of Long Living People and Centenarians and Their Siblings

In frames of the research were included 121 long living people from Bulgaria over 90 years of age have been studied together with their 294 siblings. The average lifespan of long lived people and centenarians in mountain areas is 94.1 years. For the average lifespan of long living people in plain areas the result is 90.6 years. The most adult person from mountain areas is 104 years old and for plain areas is 97 years old. For the brothers and sisters of long living people from mountain areas the average lifespan is 88.5 years. For the brothers and sisters of long live people from plain areas the average lifespan is 86.4 years. The difference in life expectancy of the two groups of people is reliable and is at $p < 0,05$, *t*-Student's. There are distances of no more than 50-70 km between these places and the only difference is mountain water and air.

The average lifespan of the long living people in the project in Smolyan municipality is 93.7 years. The oldest person is 103 years old. The lifespan of their siblings is 76.7 years. For the average lifespan of long living people in plain areas from other municipalities the result is 90.6 years. The difference of 93.7-90.6=3.1 years is essential and the project shows the reasons.

There have been 40155 residents in Smolyan municipality and 196 of them were born before 1928.

Figure 1 shows the interrelation between the year of birth of long lived people (age) and their number (Smolyan municipality, Bulgaria).

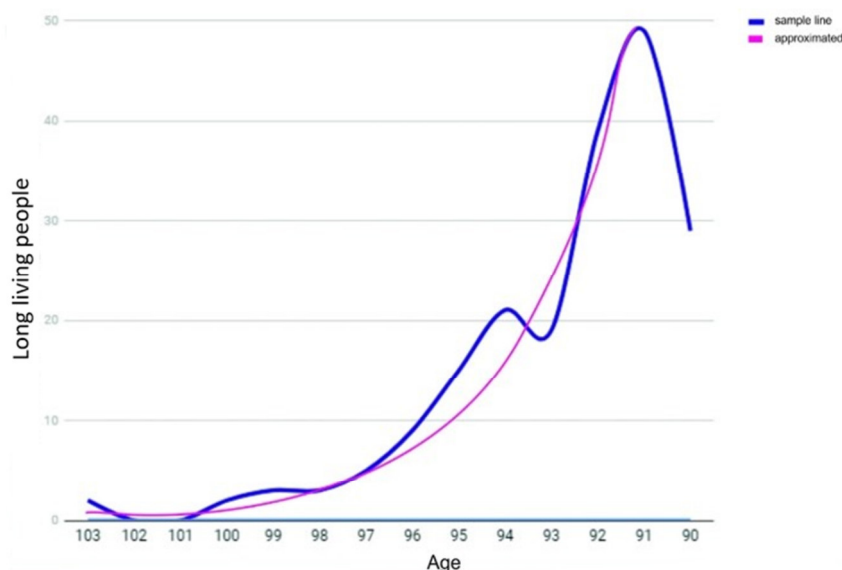


Figure 1: Interrelation between the year of birth of long living people (age) and their number in Smolyan municipality, Rhodope Mountains, Bulgaria.

The research shows that more than 90% of long living people and centenarians in Smolyan Municipality are in excellent health status. Hadzihristev gives data that more than 70% have heredity and from the data of the authors more than 80%. From the research in 6 municipalities from the regions Lovech, Pleven and Plovdiv, Bulgaria this percent is 40. On Figure 2 is done comparative analysis between the number of long-livers and centenarians, and their age in municipality of Smolyan (curve B, violet color) compared with another mountain municipality (curve A, green color). The two municipalities are with between 20 000 and 40 000 people.

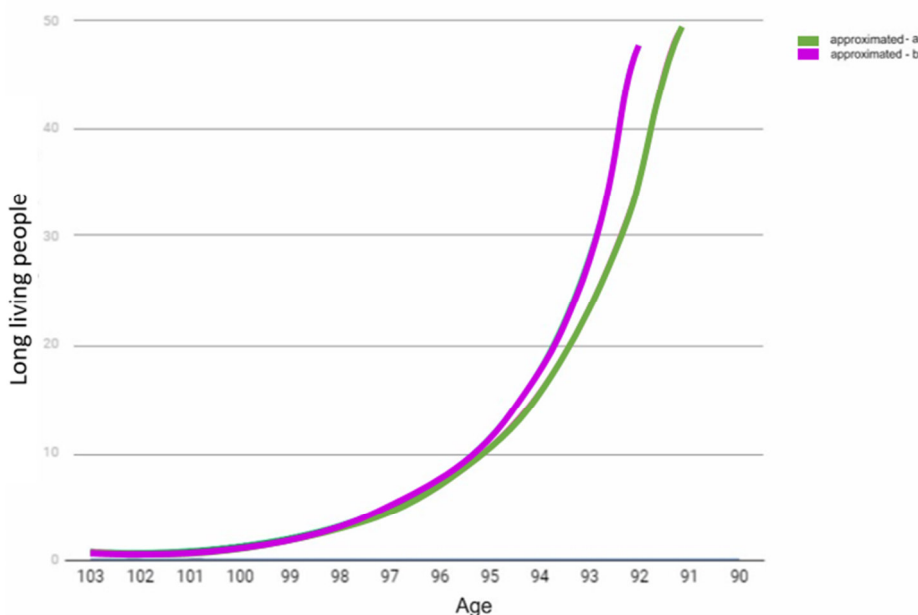


Figure 2 Comparative analysis between the number of long-livers and centenarians, and their age in Municipality of Smolyan, Rhodope Mountains (curve B, violet color) compared with another mountain municipality (curve A, green color).

The slope of the chart for long-living people in municipality of Smolyan is lesser than the one for the other mountain municipality. That is a proof for a decrease in error rate in DNA replication. It is defined by approximately 40 % higher percentage of heritable long-living people and by the environmental factors (Ignatov&Pesheva, 2018). The estimation method was created by (Ignatov & Mosin, 2014). The authors suggest that changes can be proven in gene SHC (Mooijaart, S. et al.;2004) for the long living people in Municipality of Smolyan, Rhodope Mountains.

It was shown in Figure 3 that the rate of aging increases with time. In 1963 L. Orgel showed that the aging

process is associated with the synthesis of abnormal proteins (Orgel, 1963). Figure 3 shows L. Orgel's results on the interrelation between age and number of cancer patients.

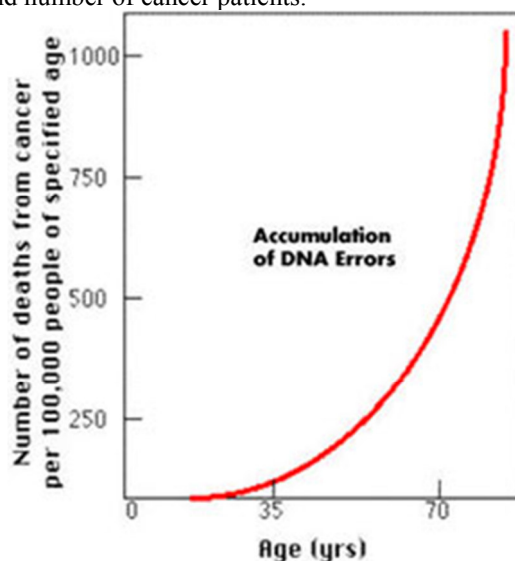


Figure 3: Interrelation between age and the number of cancer patients (Orgel, 1963).

The accumulation of errors in synthesis of abnormal proteins increases exponentially over time with age. Cells taken from elderly people show the reduced levels of transcription or transmission of information from DNA to RNA. Therefore, the probability of cancer increases with age. The interrelation between the number of Bulgarian long living people and centenarians in the mountainous municipality of Smolyan and their age is close to exponential.

Here are submitted the data for Bulgaria:

Varna district – centenarians 44 per 1 million of inhabitants, plain and sea regions;

Pleven district – centenarians 78 per 1 million of inhabitants, plain regions;

Smolyan municipality – centenarians 172 per 1 million of inhabitants, hills and mountainous regions;

Bulgaria – centenarians 35 per 1 million of inhabitants.

Analogous situation is observed in the Russian North. According to G. Berdishev, people inhabiting the Russian North – the Yakuts and the Altaians as well as the Buryats, drink mountain water obtained after the melting of ice. Altai and Buryat, Kavkaz water sources are known as moderately warm, with temperatures of 8–10 °C, the water is generally ice-free in winter. This phenomenon is explained by the fact that the melt water contains a low percentage of deuterium compared with ordinary tap water that is believed to have a positive effect on the tissue cells and metabolism. Melt water in Russia is considered to be a good folk remedy for increasing physical activity of the human body, enhancing the vitality of the organism and has a beneficial effect on metabolism (Goncharuk *et al.*, 2013). In the world are popular the sources with melt water from Austria, Switzerland, Canada, Norway, Island, Chile and Alaska. One of the coauthors (Ignatov) has performed the research of the melt glacier water from Switzerland and Chile.

3.2. Clinical Evidence with Human Blood Serum Testing

It was established experimentally that the process of evaporation of water drops, the wetting angle θ decreases discretely to 0, and the diameter of water drop basis is only slightly altered, that is a new physical effect (Antonov, 1995; Antonov & Yuskesselieva, 1983). Based on this effect, by means of measurement of the wetting angle within equal intervals of time is determined the function of distribution of H₂O molecules according to the value of $f(\theta)$. The distribution function is denoted as the energy spectrum of the water state. Theoretical research established the dependence between the surface tension of water and the energy of electromagnetic hydrogen bonds among individual H₂O-molecules (Antonov, 1995). The hydrogen bonding results from interaction between electron-deficient H-atom of one H₂O molecule (hydrogen donor) and unshared electron pair of an electronegative O-atom (hydrogen acceptor) on the neighboring H₂O molecule; the structure of hydrogen bonding may be defined as $O \cdots H^{\delta+} - O^{\delta-}$.

For calculation of the function $f(E)$ represented the energy spectrum of water, the experimental dependence between the wetting angle (θ) and the energy of hydrogen bonds (E) is established:

$$f(E) = \frac{14,33 f(\theta)}{[1 - (1 + bE)^2]^2}, \quad (1)$$

where $b = 14.33 \text{ eV}^{-1}$

The relation between the wetting angle (θ) and the energy (E) of the hydrogen bonds between H_2O molecules is calculated by the formula:

$$\theta = \arccos(-1 - 14.33E), \quad (2)$$

The energy spectrum of water is characterized by a non-equilibrium process of water droplets evaporation, therefore, the term non-equilibrium spectrum (NES) of water is used.

The difference $\Delta f(E) = f(\text{samples of water}) - f(\text{control sample of water})$ – is called the “differential non-equilibrium energy spectrum of water” (DNES).

Thus, DNES spectrum is an indicator of structural changes of water as a result of various external factors. The cumulative effect of these factors is the same for the control sample of water and the water sample being under the influence of this factor.

Figure 4 shows the average NES-spectrum of deionised water as result of one years measurements. On the X-axis are shown three scales. The energies of hydrogen bonds among H_2O molecules are calculated in eV. On the Y-axis is depicted the function of distribution of H_2O molecules according to energies $f(E)$, measured in unit eV^{-1} . For DNES spectrum the function is $\Delta f(E)$ in unit eV^{-1} . Arrow A designates the energy of hydrogen bonds among H_2O molecules, which is accepted as most reliable in spectroscopy. Arrow B designates the energy of hydrogen bonds among H_2O molecules the value of which is calculated:

$$\bar{E} = -0,1067 \pm 0,0011 \text{ eV} \quad (3)$$

Arrow C designates the energy at which the thermal radiation of the human body, considered like an absolute black body (ABB) with a temperature $36.6^\circ C$, is at its maximum. A horizontal arrow designates the window of transparency of the earth atmosphere for the electromagnetic radiation in the middle infrared range of the Sun toward the Earth and from the Earth toward the surrounding cosmic space. It is seen that the atmosphere window of transparency almost covers the energy spectrum of water.

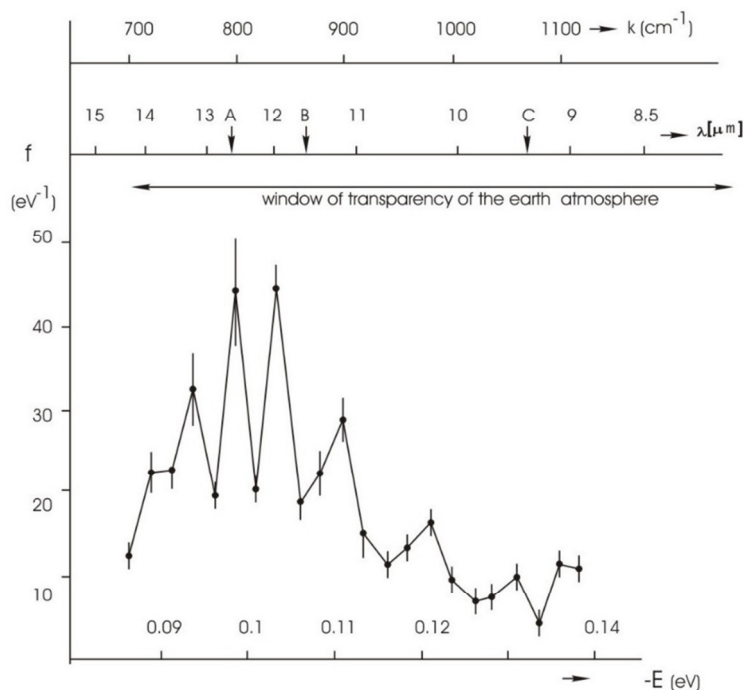


Figure 4: Non-equilibrium (NES) spectrum of water; λ – wavelength; k – wave number

The energy of the hydrogen bonding in the water dimer is 0.2 eV ($\sim 5 \text{ kcal/mol}$), which is larger than the energy of thermal motion of the molecules at the temperature of 300 K . Hydrogen bonds are easily disintegrated and re-formed through an interval of time, which makes water structure quite unstable and changeable (George, 1997). This process leads to structural inhomogeneity of water characterizing it as an associated heterogeneous two-phase liquid with short-range ordering, i.e. with regularity in mutual positioning of atoms and molecules, which reoccurs only at distances comparable to distances between initial atoms, i.e. the first H_2O layer.

In 2005 R. Saykally (University of California, USA) calculated the possible number of hydrogen bonds and the stability of water clusters depending on the number of H_2O molecules (Figure 5) (Saykally, 2005). It was also estimated the possible number of hydrogen bonds (100) depending on the number of H_2O molecules (250) in clusters (Sykes, 2007). O. Loboda and O.V. Goncharuk provided data about the existence of icosahedral water clusters consisting of 280 H_2O molecules with the average size up to 3 nm (Loboda & Goncharuk, 2010). The ordering of water molecules into associates corresponds to a decrease in the entropy (randomness), or decrease in the overall Gibbs energy ($G = \Delta H - T\Delta S$). This means that the change in enthalpy ΔH minus the change in

entropy ΔS (multiplied by the absolute temperature T) is a negative value. These results are consistent with our data on research of DNES spectrum of water on which it may make conclusion about the number of H_2O molecules in water clusters. DNES spectrum of water has energy ranges from -0.08 to -0.14 eV. The spectral range lies in the middle infrared range from 8 to $14 \mu m$ ("window" of the atmosphere transparency to electromagnetic radiation). Under these conditions, the relative stability of water clusters depends on external factors, primarily on the temperature. It was shown that the H_2O molecules change their position in clusters depending on the energy of intermolecular $H...O$ hydrogen bonds. The values of the average energy ($E_{H...O}$) of hydrogen bonds between the H_2O molecules in the formation of cluster associates with formula $(H_2O)_n$ compile -0.1067 ± 0.0011 eV. As the energy of hydrogen bonds between H_2O molecules increases up to -0.14 eV, the cluster formation of water becomes "restructuring". In this case, the energy redistribution among the individual H_2O molecules occurs towards highest energies.

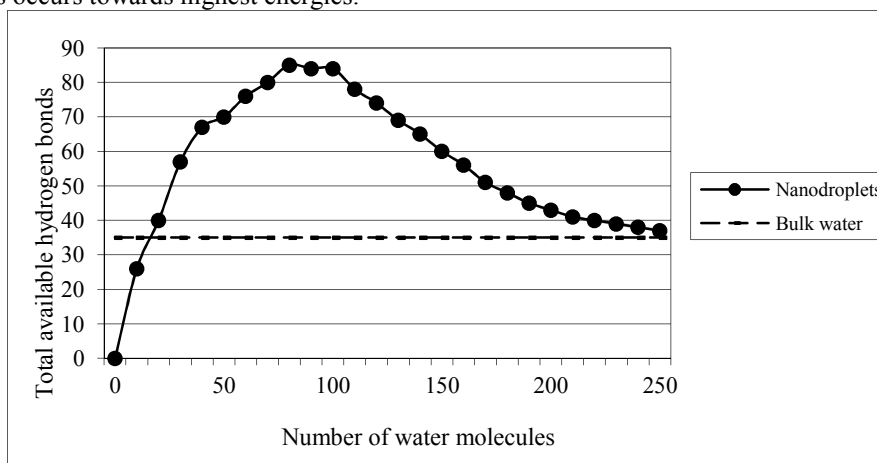


Figure 5: The total number of hydrogen bonds depending on the number of H_2O molecules in clusters.

The study of the IR spectrum of water in the composition of physiologic fluids (urine, blood, serum) can also provide data on metabolic processes in the human body and longevity, because the IR-spectrum reflects the metabolic processes. Authors have conducted studies of a 1% (v/v) solution of blood serum by spectral analysis of non-equilibrium energy (NES) spectrum and differential equilibrium energy (DNES) spectrum on two groups of people between 50 and 70 years of age. The first group consisted of people in excellent health. The second group consisted of people in a critical state and patients with malignant tumors. As the main biophysical parameter was investigated the average energy of hydrogen bonds ($\Delta E_{H...O}$) between H_2O molecules in the blood serum. The result was obtained as a difference between the NES-spectrum of 1% solution of blood serum and NES-spectrum of deionized water control sample – DNES-spectrum, measured as the difference $\Delta f(E) = f(\text{samples of water}) - f(\text{control sample of water})$. The DNES-spectrum obtained from the first group has a local maximum energy ($\Delta E_{H...O}$) at -9.1 ± 1.1 meV and from the second group -1.6 ± 1.1 meV. Results between the two groups have a statistical difference in Student's criterion at $p < 0.05$. For the control group of healthy people the value of the largest local extremum in the DNES-spectrum was detected at -0.1387 eV, or at a wavelength of $8.95 \mu m$. For the group of people in a critical state and the patients with malignant tumors, the analogous values of the largest local extremums of the DNES-spectrum shifted to lower energies compared with the control group of people. Water in the human body possesses IR-spectrum that reflects the metabolic processes in the organism. It can be demonstrated by analysis of human blood serum by IR-spectroscopy. The magnitude of the largest local extremum in IR-spectrum of blood serum from healthy people of control group observed at -0.1387 eV at a wavelength – $8.95 \mu m$. For a group of people in critical health condition and patients with malignant tumors the greatest values of local extremum in the IR-spectrum are shifted to lower energies relative to the control group. In IR-spectrum of human blood serum are detected local maxima at $\lambda = 8.55, 8.58, 8.70, 8.77, 8.85, 9.10, 9.35$ and $9.76 \mu m$ (Krasnov, Gordetsov, 2009). The resulting extremum at $\lambda = 8.95 \mu m$ in IR-spectrum (Ignatov, 2012) approaching the extremum at $\lambda = 8.85 \mu m$ monitored by Russian researchers. In the control group of healthy people the average value of the energy distribution function $f(E)$ at $\lambda = 8.95 \mu m$ compiles $75.3 eV^{-1}$, and in a group of people in critical condition – $24.1 eV^{-1}$. The level of reliability of the results is $p < 0.05$ according to Student's t-test. In 1995 A. Antonov performed DNES-experiments with impact on tumor mice cells in water (Antonov, 1995). There was a decrease of the spectrum compared with the control sample of cells from a healthy mouse. The decrease was also observed in the spectrum of human blood serum of terminally ill people relative to that of healthy people. With increasing of age of long-living blood relatives, the function of distribution of H_2O molecules according to energies at -0.1387 eV decreases. In this group of tested people the result was obtained by DNES at -5.5 ± 1.1 meV, the difference in age was of 20–25 years in relation to the control group. It should be

noted that most of Bulgarian centenarians inhabit the Rhodopes Mountains areas. Among to the DNES-spectrum of mountain waters similar to the DNES-spectrum of blood serum of healthy people at $\lambda = 8.95 \mu\text{m}$, was the DNES-spectrum of water in the Rhodopes. The mountain waters from Teteven, Boyana and other Bulgarian provinces have similar parameters. Tables 1 shows the composition of mountain springs in village of Momchilovtsi, Smolyan Municipality (Bulgaria) and local extremums in NES-spectra of water. The local extremums were detected at -0.11 eV and -0.1387 eV . The value at -0.11 eV is characteristic for the presence of Ca^{2+} . The value at -0.1387 eV is characteristic for inhibiting the growth of cancer cells. Experiments conducted by A. Antonov with cancer cells of mice demonstrated a reduction of this local extremum to a negative value. Therefore, a new parameter is entered into Tables 1– a local extremum of energy at $(-0.1362\text{--} -0.1387 \text{ eV})$. The drinking of mountain melt water structures water molecules at $\lambda = 8.95 \mu\text{m}$ and there is inhibition of development of tumor cells and there is increasing of DNA replication towards longevity. From 2014 is the new parameter and this is local extremum at $(-0.1362\text{--} -0.1387 \text{ eV})$ (Ignatov&Mosin, 2014). This value was determined by the NES-spectrum as function of distribution of individual H_2O molecules according to energy $f(E)$. The norm has statistically reliable result for human blood serum for the control group of people having cancer at the local extremum of $f(E) \sim 24.1 \text{ eV}^{-1}$.

3.3. Composition of water in the in Momchilovtsi village, Smolyan Municipality, Rhodope Mountains

This was performed analysis of water source from Momchilovtsi village (Tables 1). It shows chemical composition, hardness, local extremum in NES-spectra of water eV^{-1} at $(-0.1362\text{--} -0.1387 \text{ eV})$.

Table 1: The composition of mountain water springs in Momchilovtsi village, Smolyan Municipality, Rhodope Mountains and local extremums in NES-spectra of water

Indicators	Results of the research (mg/dm^3)	Norm
Sodium (Na)	2.7	< 200
Calcium (Ca)	65.0	< 150
Magnesium (Mg)	4.1	< 80
Potassium (K)	1.5	-
Iron (Fe)	10.0 $\mu\text{g}/\text{dm}^3$	<0.2
Manganese (Mn)	< 1.0 $\mu\text{g}/\text{dm}^3$	<0.2
Zinc (Zn)	0.007	<4.0
Sulfates (SO_4)	8.0	< 250
Chlorides (Cl)	1.5	< 250
Carbonates (CO_3)	< 2.0	-
Hydrocarbonates (HCO_3)	222	-
Other values	Results	
Active reaction (pH)	alkaline	6.5-9.5
Electroconductivity	300 $\mu\text{S}/\text{cm}$	< 2000
Hardness of water	10.09 dH Hard	<33.7
local extremum* eV^{-1} at (-0.1087--0.1112 eV)	48.0	-
local extremum* eV^{-1} at (-0.1362--0.1387 eV)	64.0	>24.1

*Function of distribution of H_2O molecules according to energy $f(E)$.

3.4. Effects of Calcium, Magnesium, Zinc and Manganese in water on biophysical and biochemical processes in the human body

Oxidative stress thus may occur in human when antioxidant mechanisms are not influence properly as in dietary deficiencies of vitamin E, vitamin C and the essential elements like selenium (Se), zinc (Zn), and manganese (Mn) among others. The later elements are essential components of the antioxidant enzymes glutathione peroxidase, superoxide dismutase and catalase. Another important cause of oxidative stress is the exaggerated endogenous production of free radicals by disease processes as in diabetes mellitus and cancer. Exposure to exogenous toxins is still another mode for inducing oxidative stress as in the toxicity of some drugs like

gentamicin or industrial chemicals like carbon tetrachloride. Apparently, then oxidative stress can be combated by strategies that promote and foster the antioxidant defense mechanisms.

The research of local extremum eV^{-1} of function of distribution of water molecules according to energy $f(E)$ at $\lambda = 8.95 \mu m$ shows characteristic for inhibiting the growth of cancer cells. In water magnesium (Mg^{2+}), zinc (Zn^{2+}) and manganese (Mn^{2+}) have influence on enzymes, which are antioxidants (Ignatov, Mosin, 2015). The research of China team was categorized three groups of elements from the rice and drinking water according to their effect on longevity: Sr, Ca, Al, Mo, and Se, which were positively correlated with longevity; Fe, Mn, Zn, Cr, P, Mg, and K, which had a weak effect on local longevity, and Cu and Ba, which had a negative effect on longevity (Lv., J et al., 2011). There was a positive correlation between eSOD activity and age and a negative correlation between eSOD activity and plasma Zn concentrations. An inverse correlation was also found between plasma Zn concentration and age. The prevalence of Zn^{2+} deficiency increased with age, with normal Zn^{2+} levels observed in about 80% of adult subjects and only in 37% of the non-agenarians. Aging is an inevitable biological process that is associated with gradual and spontaneous biochemical and physiological changes and increased susceptibility to diseases. Because nutritional factors are involved in improving immune functions, metabolic harmony, and antioxidant defense, some nutritional factors, such as zinc, may modify susceptibility to disease and promote healthy aging. *In vitro* (human lymphocytes exposed to endotoxins) and *in vivo* (old or young mice fed with low zinc dietary intake) studies revealed that zinc is important for immune efficiency (innate and adaptive), antioxidant activity (superoxide dismutase), and cell differentiation via clusterin/apolipoprotein J. Intracellular zinc homeostasis is regulated by metallothioneins (MT) via ion release through the reduction of thiol groups in the MT molecule (Mocchegiani, E. 2007). Zinc from water improves antioxidant enzymes in red blood cells (Malhotra, A., Dhawan, D. K., 2008).

Magnesium deficiency and oxidative stress have both been identified as pathogenic factors in aging and in several age-related diseases. The link between these two factors is unclear in humans although, in experimental animals, severe Mg deficiency has been shown to lead to increased oxidative stress (Begona, M. et al, 2000). Defenses against free radical damage include tocopherol (vitamin E), ascorbic acid (vitamin C), beta-carotene, glutathione, bilirubin, and several metalloenzymes including glutathione peroxidase (selenium), catalase (iron), and superoxide dismutase (copper, zinc, manganese) and proteins such as ceruloplasmin (copper). The extent of tissue damage is the result of the balance between the free radicals generated and the antioxidant protective defense system (Machlin, L. J., Bendich, A, 1988). The norm in water for zinc and manganese from World Health Organization (WHO) is less than $20 \mu g/dm^3$. For the Sodium (Na) the norm from WHO is less than $20 mg/dm^3$.

There were results of USA and Canada for the concentration of calcium (Ca) in water. From statistical information the most centenarians in Canada per million are in Nova Scotia (210 per 1 million). In the water from Nova Scotia calcium is 6.8 mg/l. The results of Nikolay Druzhyak were from different places in Russia. He shows that in the places with centenarians the calcium is between 8 and $20 mg/dm^3$. In the report we were shown the dependence of antioxidant effects and longevity. Risk factors are cardiovascular diseases.

The following reactions occur in water if there are high concentrations of calcium and magnesium ions: The reaction of limestone ($CaCO_3$) and gypsum ($CaSO_4 \cdot 2H_2O$) with water to separate the calcium Ca^{2+} , carbonate CO_3^{2-} and sulfate ions SO_4^{2-} . By increasing the mineralization the content of Ca^{2+} ions decreases. During the concentration of the solutions they were precipitated. With the increase of carbon dioxide CO_2 and decreasing of pH increases the concentration of Ca^{2+} . Reaction of dolomite ($CaCO_3 \cdot MgCO_3$) with water makes the formation of magnesium Mg^{2+} ions. Magnesium salts are well soluble in water. Hydrocarbonates (HCO_3^-) and carbonates (CO_3^{2-}) ions are formed by reaction of the karst rocks, carbon dioxide and water. There was separation of carbonic acid (H_2CO_3). There was observation of antioxidant effects of water on rats (Abdullah, A.M, 2012). In Zamzam water there is $Ca^{2+} - 299.7$; $Mg^{2+} - 18.9$; $Zn^{2+} - 0.001 mg/l$.

3.5. Results for oxidant/antioxidant balance

The research shows that over 90% of long-livers and centenarians in the municipality of Smolyan are in an excellent state of health. According to data by Hadzhihrstev over 70% have heredity, and according to the author-over 80%. During research of 6 municipal regions in Lovech, Plevan and Plovdiv the percentage is 40%. Which are the factors that determine an excellent balance of oxidants/antioxidants in the Rhodopes Mountains?

1. Zinc (Zn)- it improves the properties of antioxidant enzymes in the blood red cells. Zinc is contained in the mountain spring water and in the following foods – meat, red beans, fresh milk and yoghurt. Research shows that zinc in healthy long-livers and centenarians is within the normal levels. The inflammations are less with them as one of the reasons for ageing.

2. Manganese (Mn) also improves the qualities of antioxidant enzymes in the red blood cells. Manganese is contained in the mountain spring water, and in the following foods – herbs (thyme, mint), parsley, rye and wheat, blueberries.

The altitude of Smolyan and surrounding villages is over 1000 m. A higher number of red blood cells are being

formed. Superoxide dismutase (SOD) is an enzyme, which counteracts one of the most dangerous ROS (Reactive Oxygen Species) radicals O_2^- . That enzyme contains ions of manganese (Mn^{2+}). The concentration of zinc (Zn) is connected with SOD and decreases with the age.

3. Magnesium (Mg) is a co-factor in enzymatic reactions. It also neutralises one of the most dangerous radicals – the hydroxyl radical OH. Rich in magnesium foods are – nuts and seeds, beans, dairy products. By neutralisation of hydroxyl radical is lowered the likelihood of depressions, Alzheimer, Parkinson disease etc.

4. The main function of calcium (Ca) is its participation in the human bone structure. Among the bone system, blood and the rest of the body parts, there is a constant circulation of that element, which is precisely controlled by the hormones. The calcium links to an extensive circle of proteins changing their biological activity. That is important for nerve impulses transmission and muscle shrinking.

Calcium is contained in mountain spring water and the following foods – milk and dairy produce, beans.

During acidification, in order to keep the alkaline pH of the blood, are being released calcium and magnesium. With the age the blood viscosity gets higher or simply said “it gets thicker”. That is a reason for cardio-vascular diseases. When consumed straight from the source, the mountain spring water saturates the body with antioxidant negative charges.

6. The balance in nervous and cardio-vascular systems gets supported mainly by potassium (K) and sodium (Na). Long lived people in the mountains feed on high in potassium foods – beans, spinach, potatoes, yoghurt, mushrooms and courgettes. Sodium is contained mostly in cheese, potatoes and certain meats.

6. Herbal teas are consumed on a regular basis.

In the municipality of Smolyan, Rhodopes Mountains is observed higher consumption of yoghurt and milk products, beans. The settlements are in the mountain without any industrial pollution. The localities are 1000 m above the sea level, and the people live in deciduous and coniferous forests with fresh mountain air with negative charges. All long-livers and centenarians report regular walks in the mountain. All of them have kept the same body weight during their entire lives. There are none of them that are overweight.

3.6. Bulgarian yoghurt with *Lactobacillus bulgaricus*.

Yoghurt has probiotic effects in digestive system on the base of *Lactobacillus bulgaricus*. Part of the intestinal mucosal barrier function is formed by a common mucosal immune system which provides communication between the different mucosal surfaces of the body. The intestinal colonisation with a balanced microflora is of main importance for the correct development of the immune system. The use of probiotics or prebiotics to correct this imbalance and modulate the immune activity. The inflammations are part of aging process. Bulgarian yoghurt influences decreasing inflammations with anti aging effect (Ignatov, Pesheva, 2018).

4. Conclusion

Interviews have been conducted with 415 Bulgarian centenarians and long living people and their siblings. 2012-2017. In 2018 in Smolyan Municipality were studied 62 long living people and centenarians and their siblings. The participants in project are 477. The siblings have the same heredity, but have lived under different conditions. Research conducted by us shows that the direct relationship of man and nature – clean air, natural food from eco-farms and physical activity explains the difference between the larger number of long living people and centenarians who live in the mountain regions of Bulgaria and their high average number. Natural mountain and melt water with chemical composition, less deuterium seems to be one of the most important factors for longevity. In Bulgaria, most long lived people and centenarians live in the Rhodope Mountains.

It worth to note that IR-spectrum of mountain water is most similar to the IR-spectrum of blood serum of healthy group of people with a local maximum at $\lambda = 8,95 \mu m$. Similar spectral characteristics possess mountain water from Smolyan and other Bulgarian sources. Thus, the phenomenon of longevity is a complex phenomenon involving both genetic and phenotypic characteristics of the organism to external factors and environment – free radicals, radiation, heavy isotopes, as well as the structure and the isotopic composition of drink water. Other longevity factors are living area, health status, body mass, gender and heredity. Studying of human blood serum by NES and DNES-methods show that by measuring the average energy of hydrogen bonds among H_2O molecules and the distribution function of H_2O molecules on energies it is possible to show a vital state status of a person and associated life expectancy. These data indicate that water in the human body has the IR-spectrum resembling IR-spectrum of human blood serum. On the characteristics of the IR-spectrum of water exerts an influence also the presence of deuterium. In the research there is optimal composition of mountain and melt water from areas where were lived the long living people and centenarians. There are new proofs for biophysical and biochemical effects of calcium (Ca), magnesium (Mg), zinc (Zn) and manganese (Mg). They also have anti-inflammatory effect. Inflammations are the shortcut to ageing. The balance is even stronger together with the natural antioxidants – fruit and vegetables with vitamins C and E. When the balance is disrupted the immune system experiences difficulties. That is quite valid if we take food of low quality, as the immune system doesn't separate between healthy and ill cells. In this way the auto-immune processes get accelerated. The balance in our

nervous and cardio-vascular system is supported mainly by potassium (K) and sodium (Na). Longer living people in the mountains consume foods rich in potassium – beans, spinach, potatoes, yoghurt, mushrooms, pumpkin and courgettes. The sodium is contained mostly in the cheese, potatoes and some meats. There are unique plant-derived products, which provide vital vitamins and minerals. In the modern world, due to its deteriorated quality, the food is not sufficient. The main question is how to choose products with easily absorbed vitamins and minerals. The magnesium can neutralise one of the most dangerous radicals – the hydroxyl radical OH. Mountain water can give you up to 7 more years of life due to its composition, arrangement of water molecules being close to the water in the body of long lived people, crystals in spring time providing more energy. That difference is gained from the living in the mountains centenarians and longer lived people in comparison with those living in the plains. The authors establish based on a spectrum analysis of blood serum of healthy people and people having cancer, which waters are of highest quality for prolonged life expectancy. Such waters increase the energy among the water molecules and sustain a healthy and stable body. In these waters the development of tumour cells in number and size is being suppressed. How can we prepare quality water, similar to mountain or glacier water? All biochemical processes in the body are in water medium. The stronger the energy of the hydrogen bonds among the water molecules, the healthier the person is. The natural medium in the human body is the alkaline medium. When a person is born its body contains around 75% of water, and it is 55-60% in adults. The conclusion comes naturally that the person has to drink water of high quality. During acidification, in order to keep the alkaline pH of the blood, are being released calcium and magnesium. With the age the blood viscosity gets higher or simply said it “gets thicker”. That is a reason for cardio-vascular diseases. The alkaline water catholyte acquired by electrolysis “dilutes” the blood. And also, similar to the mountain water that is consumed straight from the source, it saturates the body with beneficial antioxidant negative charges. For the modern man the acidification is easier compared to our predecessors, and therefore the alkaline waters are vital necessity. There are also natural minerals with unique properties as the water goes through the Earth layers.

The failure to comply with these conditions can bring imbalance, difficulties for the immune system to cope and lowered life vitality. The path to illnesses is short. During their life humans get older and this is quite a natural result of the evolution. The DNA replication from one mother cell to two daughter cells is exponential. Lesser the faults, longer the life of the person. The balance is decisive for the slower ageing.

The research shows that more than 90% of long living people and centenarians in Smolyan Municipality are in excellent health status. The data of По данни на Хаджихристов над 70% имат наследственост, а според данни на автора над 80%. При изследване в на 6 общини области Ловеч, Плевен и Пловдив този процент е 40.

There is proof for less errors in DNA replication. It is defined by approximately 40 % higher percentage of heritable long-living people and by the environmental factors (Ignatov&Pesheva, 2018). The estimation method was created by (Ignatov & Mosin, 2014). The authors suggest that changes can be proven in gene SHC.

The basic conclusion is that the balance oxidants/antioxidants for the long living people in Smolyan Municipality (Ignatov&Pesheva, 2018).

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