ОБЩА МЕДИЦИНА
GENERAL MEDICINE

Published in:
Journal of General Medicine,
Bulgaria; 2;2013, pp 21-30

EFFECTS OF ARONIA MELANOCARPA FRUIT JUICE IN IMPROVING MEDICAL TEST RESULTS AND CREATING A FEELING OF HEALTH IN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE – NAFLD (steatosis)

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Introduction

Non-alcoholic steatosis disease of the liver (NAFLD) is a clinico-morphological concept. It occurs when accumulation of fats (triglycerides) is observed in at least 10% of hepatocytes in people who do not consume toxic amounts of alcohol (630 ml spirits a week, 2100 ml of wine a week, or 4200 ml of beer a week). NAFLD includes steatosis, non-alcoholic steatohepatitis, and cirrhosis. This is the most common cause for elevated serum aminotranspherases not associated with hepatitis viral infections. There is enough evidence to support the view that NASDL presents the liver manifestation of metabolic syndrome (4).

In 1988, Reavon introduces the concept of "Syndrome X" to emphasize that insulin resistance and subsequent hyperinsulinemia predisposes patients to hypertension, hyperlipidemia, and diabetes mellitus and develops against the background of abdominal fat accumulation. Some other characteristics of the same syndrome are the presence of proinflammatory condition, microalbuminuria, and hypercoagulation. To assess this constellation, the term currently used is "metabolic syndrome" (MS).

Since times immemorial the fruits of aronia melanocarpa have been known for their medicinal properties. They have been used in the treatment of various diseases and have invariably shown effects which enhance the healing process. In non-traditional medical practices, aronia fruits have found application in the treatment of achlorhydria, cases of avitaminosis, during the re-convalescence stage of serious diseases, and, last but not least – in hemorrhoids treatment. Present-day research of pharmacological effects from aronia melanocarpa juice and fruits use indicate that their high contents of anthocyanins is closely related to the health enhancing properties typical of this plant. This is a key fact which can be used in the prevention

of most commonly spread, socially significant diseases through reducing the total risk of cardio-vascular diseases, the lipidomodifying activity the plant shows, and the anti-mutagenic effects it demonstrates (9,2). The anthocyanins taken orally, and then finding their way to the gastro-intestinal tract are sensitive to the pancreas digestive medium which has an alkaline nature. The latter is associated to modification in the biopresence of anthocyanins. On the other hand, there is evidence that the nature of aglycons and saccharides is relevant to the chemical stability, absorption, and metabolism of anthocyanins. This is of major importance when defining their *in vivo* activity (16). In research employing people it is difficult to investigate for tissue biopresence (which is a well-studied area with experimental animals) of the anthocyanins taken – given the fact that they distribute and act on various sites considering the needs (15). The great molecular variety anthocyanins possess, the part they play in cells metabolism which is still being investigated, set the target to study *the effects* on the cells, tissues, and organs, *as a response to the intake* by following the adequate laboratory markers of cells and organs (Talbe 8)

Table 8. Chemical composition of the fruit of aronia melanocarpa.

Chemical component	Quantities and Units
Fruit water	65 – 72%
Dry matter content	0.44% of fresh weight
Organic acids	1.1 – 1.4%
Tannins	0.5 – 0.6%
Polyphenols (Vit.P)	2000 – 8000mg/100g dry matter
Carbohydrates	10 – 18%
Pectin	0.5 – 0.6%
Sorbitol and parasorbid	Including in the percentage of carbohidrates
Fet:Linoleic acid, Glycerides,Phosphatidilinositol	0.14% of fresh weight
Minerals: K, Zn, Na, Ca, Mg, Fe.	Micro quantities
Vitamins: B1, B2, B6, B9, K, E, C, niacin, pantothenic acid, folic acid, α- and β-tocopherols, carotenoides.	Various concentrations sufficient as a daily dose (mg/%)
Amminoacids:	(in mg/dl)
Arginine	65
Tyrosine	38.6
Histidine and lysine	62.2
Cystin	17.4
α-alanine	12.2
Asparginate and serine	14.7
Glutamate and treonine	9.3
Micronutrients	(µg/%)
Jodine	4000
Manganese	500
Boron	Traces
Molybdenum	Traces
Cuprum	Traces
Tripertens: β-sitosterol, campesterol	Micro quantities
Over 40 volatiles: benzaldehide cyanohydrin, hydrocianids acid, benzaldehide.	Traces
Amigdalinum (Vit B17)	Traces

Bulgaria is a country which has favourable climatic conditions, soil, and precipitation levels for the planting and growth of Aronia melanocarpa. Specifically, the sun-shine hours in our latitude allows for ripening of fruits having the highest contents of useful substances in comparison to other European countries (3). The beginning of biological growth of aronia melanocarpa in this country dates back to

1995. The presence in Bulgaria of EC-certified bioproducers of aronia products (juice, fruit wine) has also impacted the decision to conduct this study.

The goal of the current study is to follow the effects of applying Dr. Barry Sears' "The Zone" Diet in combination with aronia melanocarpa juice intake on patients having laboratory, clinical, and sonographyc dates of liver steatosis, as a representative of NAFLD.

Materials and methods

Twenty-five patients (18 men and 7 women) aged 46 to 56, have been selected for the research in the process of prophylactic check-ups that included liver enzymes analysis. Following exclusion of hepatitis B and hepatitis C virus, they were referred to abdominal and liver sonography. In this way the NAFLD-steatosis clinical diagnosis group was selected. The patients were introduced to Barry Sears' "Zone Diet" methods of life, which 18 of them (15 men and 3 women) complemented with aronia melanocarpa juice intake[1] in a dose of 200–250 ml/day divided in three portions 20 minutes before meals over a period of 60 days. The amount of aronia juice for one portion is diluted by an equal amount of water and after that the juice of half a lemon is added. The drink is taken in slowly for about 5-6 minutes. Another 7 NAFLD-steatosis patients are to be used as a control group to compare the medical investigations results. They will be given aronia melanocarpa juice for the first time on day 60 of the treatment course. The patients were subjected to laboratory analysis including parameters of hepatic, carbohydrate, and lipid exchange, in specific: ASAT, ALAT, GGT, basic blood glucose, basic insulin, HOMA-IR, HвA1c; total cholesterol, HDL-cholesterol, LDL-cholesterol, ApoA, and ApoB. The above mentioned indicators have been analyzed when diagnosing the subjects input values, late on day 30-th, and late on day 60-th. The patients also completed an enquiry form – a direct type with open questions to reflect changes in their general health status, as well as answering leading questions referring to specific organs and systems. It should be noted here that the patients involved in the study are erudite respondents. Laboratory analyses have all been done on ABBOTT highly automated and robot platforms using chemical and immune-chemical methods type A for the respective parameters, in laboratories which pass systemic internal and external quality results assurance. Blood samples for analysis have been collected by venipuncture between 8:00 a.m. and 8:30 a.m. before meals.

Results were statistically processed by SPSS non-parametric descriptive analysis.

Results and discussion

Data collected on following the adequate laboratory parameters is given in tables 1, 2, 3, 4, 5, 6, and 7. Patients on aronia melanocarpa juice course have shown changes in their laboratory results as follows: from input values of ASAT, ALAT, and GGT (mean values expressed in IU/L) correspondingly 181±16, 163±21,and 487±77, the values (mean ones again) in the end of the second month became 21±11, 38±6, and 63±9 for the respective parameter (Table 3).

[1] Produced by "Aroniada Agro" Ltd – Bulgaria, production year 2011

Table № 3. Changes in blood serum activity of mine liver enzymes in patients with Barry Sears lifestyle and with aronia juice intake (mean values ± SD).

Laboratory parameters	Basal values	Values after 30 days	Values after 60 days
ASAT (IU/L – 37oC)	181 ± 16	91 ± 13	21 ± 11
ALAT (IU/L – 37oC)	163 ±2 1	88 ± 16	38 ± 6
GGT (IU/L – 37oC)	312 ± 77	189 ± 65	63 ± 9

(p<0.05)

The indices for carbohydrate exchange have improved: basal blood glucose level of 7,15±0,07 (mmol/L) , basal insulin level of от 14,4±1,95 (mIU/L), HOMA-IR of 3,29±1,02, and HвA1c of 6,97±0,11(%) in the beginning of treatment course have changed in the end of the second month to the following values respectively: 5,12±0,08, 7,5±1,07, 1,73±0,85, and 5,17±0,08 (Table 2).

Table № 2. Changes in blood concentrations of some laboratory parameters of carbohydrate metabolism in patients with Barry Sears lifestyle and with aronia juice intake (mean values ± SD)

Laboratory parameters	Basal values	Values after 30 days	Values after 60 days
Fasting blood glucose (mmol/l)	7,15±0,07	6,05±0,46	5,12±0,08
Basal insulin (mIU/L)	14,4±1,95	9,37±1,16	7,51±1,07
HOMA-IR	3,29±1,02	2,45±0,97	1,73±0,85
HbA1c (%)	6,97±0,11	-	5,17±0,08

(p<0.05)

Favourable changes have also been observed in the laboratory markers for metabolism of fats. Before treatment application total cholesterol level was 6,8±0,15(mmol/L), HDL-cholesterol – 0,89±0,12, LDL-холестерол – 5,29±0,11; triglycerides – 3,93±0,25(mmol/L); ApoA – 0,84±0,13(g/L); ApoB –2,23±0,15(g/L). In the end of the second month the levels were respectively: 4,95± 0,03, 1,41±0,05, 3,02±0,08, 1,01±0,03, 2,03±0,06, and 0,85±0,07 (Tables 1).

Table 1. Changes in blood concentrations of some laboratory parameters of lipid metabolism in patients with Barry Sears's lifestyle and with aronia juice intake (mean values ± SD)

Лабораторен параметър	Basal values	Values after 30 days	Values after 60 days
Total Cholesterol(mmol/l)	6.8 ± 0.15	5.85 ± 0.09	4.95 ± 0.03
HDL-cholesterol (mmol/l)	0.89 ± 0.12	1.05 ± 0.13	1.41 ± 0.05
LDL-cholesterol (mmol/l)	5.29 ± 0.11	4.36 ± 0.13	3.02 ± 0.08
Triglycerides (mmol/l)	3.93 ± 0.25	1.93 ± 0.17	1.01 ± 0.03
Apo A1 (g/l)	0.84 ± 0.13	0.96 ± 0.09	2.01 ± 0.06
Apo B (g/l)	2.23 ± 0.15	2.01 ± 0.08	0.83 ± 0.07

(p<0.05)

On comparison with the values of a control group of seven people (Tables 4, 5, 6) who kept the rules of a Barry Sears lifestyle, but did not take aronia melanocarpa juice, the values above are significantly better, they also fit the reference range for all investigated parameters (Table 7 and Diagram 1).

Table 4. Changes in blood concentrations of some laboratory parameters of lipid metabolism in patients with Barry Sears lifestyle and without aronia juice intake (mean values ± SD)

Лабораторен параметър	Basal values	Values after 30 days	Values after 60 days
Total Cholesterol(mmol/l)	7.12 ± 0.30	6.48 ± 0.13	5.85 ± 0.10
HDL-cholesterol (mmol/l)	0.78 ± 0.18	0.84 ± 0.19	1.04 ± 0.12
LDL-cholesterol (mmol/l)	5.74 ± 0.116	4.50 ± 0.21	4.21 ± 0.13
Triglycerides (mmol/l)	3.06 ± 0.35	2.02 ± 0.19	1.78 ± 0.09
Apo A1 (g/l)	0.87 ± 0.17	0.93 ± 0.12	1.24 ± 0.05
Apo B (g/l)	2.09 ± 0.19	1.92 ± 0.16	1.15 ± 0.21

($p<0.05$)

Table № 5. Changes in blood concentrations of some laboratory parameters of carbohydrate metabolism in patients with Barry Sears's lifestyle and without aronia juice intake (mean values ± SD).

Laboratory parameters	Basal values	Values after 30 days	Values after 60 days
Fasting blood glucose (mmol/l)	7,21 ± 0,11	6,12 ± 0,75	6,00 ± 0,32
Basal insulin (mIU/L)	15.3 ± 2,12	10.95 ±1 ,93	9.01 ± 0,87
HOMA-IR	3,47 ± 1,23	2,83 ± 0,89	2,45 ± 0,15
HbA1c (%)	7,03 ± 0,12	-	6,23 ± 0,17

($p<0.05$)

Table № 6. Changes in blood serum activity of mine liver enzymes in patients with Barry Sears lifestyle and without aronia juice intake (mean values ± SD).

Laboratory parameters	Basal values	Values after 30 days	Values after 60 days
ASAT (IU/L – 37°C)	176 ± 19	117 ± 10	99 ± 13
ALAT (IU/L – 37°C)	158 ± 19	109 ± 11	87 ± 15
GGT (IU/L – 37°C)	299 ± 69	142 ± 41	109 ± 21

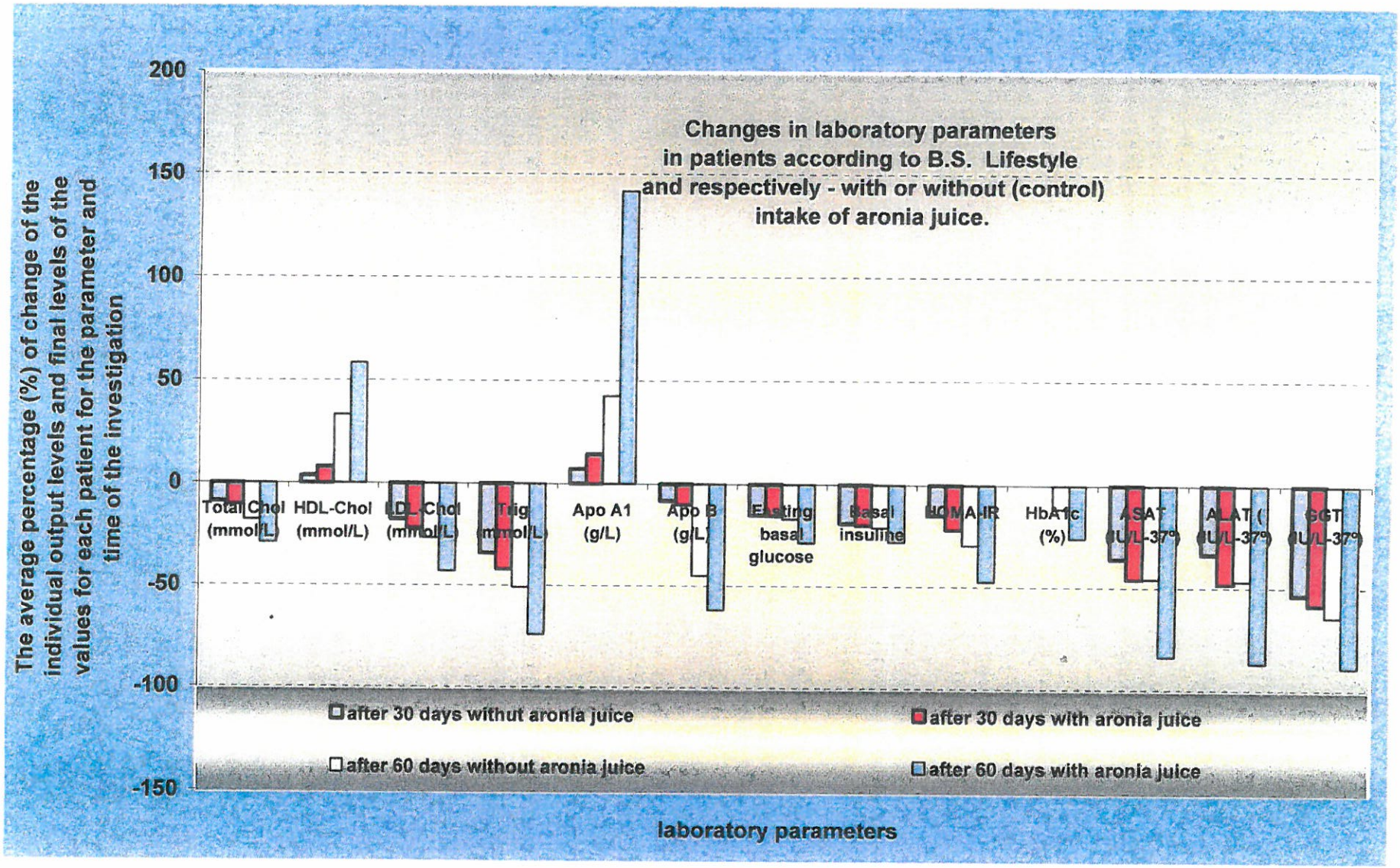
($p<0.05$)

Table 7. Changes in laboratory parameters in patients according to Barry Sears lifestyle and respectively - with or without (control) intake of aronia juice. The results are present as the average percentage (%) of changes of the individual output levels and final levels of the values for each patient and each parameter and time of the investigation.

Laboratory parameters	Values after 30 days without aronia juice intake	Values after 30 days with aronia juice intake	Values after 60 days without aronia juice intake	Values after 60 days with aronia juice intake
Lipids				
Total Cholesterol (mmol/l)	-8,99	-10,46	-17,8	-29,1
HDL-cholesterol (mmol/l)	3,37	7,7	33,3	58,4
LDL-cholesterol (mmol/l)	-17,5	-21,6	-26,6	-42,9
Triglycerides (mmol/l)	-34,0	-41,8	-50,9	-74,3
Apo A1 (g/l)	6,9	14,3	42,5	141,7
Apo B (g/l)	-8,1	-9,86	-44,9	-61,9
Carbohidrates				
Fasting blood glucose (mmol/l)	-15,1	-15,4	-16,8	-28,4
Basal insuline (mIU/L)	-18,8	-19,5	-20,8	-27,8
HOMA-IR	-14,9	-21,5	-29,4	-47,4
HbA1c (%)	-	-	-11,38	-25,8
Mine liver enzymes				
ASAT (IU/L – 37oC)	-35,7	-45,8	-45,6	-84,0
ALAT (IU/L – 37oC)	-33,12	-48,1	-46,6	-87,1
GGT (IU/L – 37oC)	-53,7	-58,5	-64,5	-89,5

See the same changes in diagram 1.

Diagram 1.


Changes in laboratory parameters
in patients according to B.S. Lifestyle
and respectively - with or without (control)
intake of aronia juice.
The average percentage (%) of change of the individual output levels and final levels of the values for each patient for the parameter and time of the investigation
200
150
100
50
0
-50
-100
-150
Total Chol (mmol/L)
HDL-Chol (mmol/L)
LDL-Chol (mmol/L)
Trig (mmol/L)
Apo A1 (g/L)
Apo B (g/L)
Fasting basal glucose
Basal insuline
HOMA-IR
HbA1c (%)
ASAT (IU/L-37°)
ALAT (IU/L-37°)
GGT (IU/L-37°)
after 30 days withut aronia juice
after 30 days with aronia juice
after 60 days without aronia juice
after 60 days with aronia juice
laboratory parameters

These changes in laboratory parameters were evidence of improved liver function. The reasons are: 1) overcome the insulin resistance of cells. 2) upgraded synthesis function of the liver cells. The liver promoted synthesis of antiatherogenic apolipoprotenis A. It increases the levels of HDL-cholesterol in blood. Significant differences in improvements in patients complemented with aronia melanocarpa juice intake and those who do not accept juice and literature data refer to favorable therapeutic effects of chokeberry juice.

Weight reduction at an average of 5±1,82 kg was recorded for all patients, and this was mainly due to the fat tissue reduction. The control ultrasound and clinical investigations, carried out at day 60, showed absence of the findings characteristic of steatosis. In the first stage of the study, 83% of the patients (21 people) were given one medication with the purpose to control their hypertension. Enquiry data show that 20% out of them (4 people) have reached the end of the treatment course with normal blood pressure values, and the others have reduced the medication dose to ½ by the time of the enquiry. All patients report improvement in various conditions that have caused them discomfort before the research started: pains in the muscles and joints faded away of and were replaced by a new feeling of strength, headache attacks disappeared, improved memory and sleep were reported, regular defecation, no signs of gastric discomfort, better eyesight, a quicker auditory reaction, motivation for having sex, good mood disposition. In comparison to the group which does not take aronia melanocarpa juice the individual perception of feeling healthy is significantly better.

The diagnostic criteria for MS have been proposed in NCEP-ATP III reports (2001), and detailed later by IDF (2005). MS is closely related to other clinical and laboratory lesions correlating to insulin resistance, some of which favor the development of cardio-vascular diseases. For instance, elevated levels above the upper reference value for: hs-CRP; LDL-cholesterol (small particles) linked to high apoB100 (apolipoprotein B100); the inhibitor to plasminogen (PAI-1) activator; ferritin, homocysteine, GGT, serum uric acid. We find values below the lower reference limit for the sex-hormone binding globulines (SHBG); microalbuminuria. There exists ample evidence in support of the relation between MS and NAFLD, polycystic ovarian disease, and the sleep apnea.

It is important to pay attention to stress. Apart from enhancing the obesity process, psycho-social stress also activates the sympatico-adrenal system (SA) and the pituitary-adrenal system. Both of them possess a strong hypertensive potential. Furthermore, the adrenal hormones released in a state of stress increase the production of fatty acids from the fat tissue and the insulin resistance, as well as the glucose production from the liver. That is why, stress acts as an additional engine for metabolic impairment caused by obesity, and in a series of cases stress is so high that it can cause insulin resistance on its own, and even lead to type 2 diabetes. This mechanism is described as insulin resistance in normal weight patients. Engaging the psyche of a person living in a stressful environment with duties that relate to his/her own self – diet, physical exercises, aronia juice intake – is a predisposing factor to relieve stress and accept the stress-inducing factors with a composed mentality.

The output data of the subjects studied, as well as the clinical findings, have values which characterize the metabolic syndrome – dyslipidemia with hypo HDL-cholesterolemia and hypertriglyceridemia, impaired carbohydrate tolerance with data on insulin resistance, elevated liver enzymes values in constellation indicative of steatosis, high percentage of fatty tissue (more than 35% of overall body weight). The risk factors for metabolic syndrome manifestation develop over the years and

associate to the oxidative stress of the cells. As known, the metabolism of fats and carbohydrates is closely connected to the liver function (6). The reverse is also true. The systemic exogenic disorders (bad eating habits, sedentary lifestyle, harmful factors exposure) the so called "bad habits" nursed for years on end bring about the imbalance in the carbohydrate and lipid metabolism through the accumulation of proinflammatory molecules – cytokines, which activate the oxidative processes in cells and cause direct damage of the most sensitive tissue – the hepatic parenchyma (5).

A specific role in NAFLD pathogenesis is played by the so-called "oxidative" stress of the cells. It occurs when the speed of formation an accumulation of ROS (reactive oxygen species) exceeds the capacity of the cell to neutralize them. O_2 (the oxygen molecule) is essential for the life of people, at the same time, it is toxic, too. Humans need the O_2 flow for the oxidizing reactions along the biochemical pathways to obtain ATP (adenosine 3-phosphate – the chemical energy source), in the detoxication processes and in biosynthesis. But when O_2 accepts free electrons, it transforms to highly reactive oxygen radicals and damages the cellular lipids, protein molecules, and the DNA. These damages contribute to the death of cells and cause a number of disorders. These oxygen radicals are also called "reactive oxygen species" – ROS. They are superoxide - O_2^-; hydrogen peroxide - H_2O_2, and hydroxyl radical -OH•. The hydroxyl radical in combination with nitrogen oxide forms another class of reactive nitrogen oxygen species - HOCL (RNOS). The intimate mechanism of intracellular damage is a complex one, but it is nowadays well documented (10, 12). The hydroxyl radical causes oxidative damage to proteins and DNA. It also forms the lipid peroxides and the malonaldehide in the membrane lipids, which contain polynonsaturated fats. In some cases, the free radical damage is the *direct* cause of a disorder – such as direct tissue damage when there is exposure to ionizing radiation. In other cases, ROS may *keep the damage brought about by other processes* – such as, neurodegenerative damage in Parkinson's disease, or insufficient blood flow to the brain or other tissues. RNOS are, on the one hand, represented in the environment (cigarette smoking), on the other hand – they form in the cells. During the phagocytosis of invading pathogenic microorganisms, the immune system cells form O_2^- , HOCL and OH• by the activity of enzymes HADPH-oxydase, myeloperoxidase, and the induced nitric oxidesynthase. ROS kill the phagocyted microorganisms, but they can in addition harm the surrounding tissue. It is therefore crucial to begin treatment of any bacterial or viral invasion well on time and take it on till the stage of full recovery combining it with good hygiene and adequate diet on the part of the patient. Sites which are only partly cured or repaired subjected to a number of other risk factors can become a target for malignant cell formations (13).

As a well-organized structure, the cell succeeds *in defending* itself from the damage induced by ROS and other radicals. This occurs through sophisticated but rapid repair processes: compartmentalization of free radicals products, defense enzymes and endogenous and exogenous antioxidants (free radical scavengers). The defense enzymes include: superoxide dismutase (SOD), catalase, glutathione peroxidase, and glutathione reductase. Non-enzyme antioxidants include: vitamin E, vitamin C, carotenoids, and **plant flavonoids** (the fruits of aronia melanocarpa having the highest content). They can be classified as exogenous antioxidants which normally react with ROS in a non-enzyme way.

To complete the information, we'll mention the major *sources* of primary ROS in the cell. There are three of them:

1. A great part of superoxides are coenzyme Q generated in the mitochondrial electron-transporting chain.

2. Enzymes in the cells: oxidases, oxygenases, and peroxidases. These enzymes bind oxygen in the cells and transfer single electrons – cascade reduction processes. Before reduction is over free radicals may be released as intermediates. For example: cytochrome P450 enzyme is a major source of free radicals. This enzyme is induced by alcohol, medications, and toxic chemical substances and may cause necrosis of the liver. Hydrogen-peroxidases and lipid-peroxidases obtained as intermediate products in eicosanoids exchange – leukotrienes and prostaglandins (molecules of inflammation) generate as well. The xantinoxidase, as an enzyme derived from purine breakdown acts in cytosol. It facilitates the degradation processes after ischaemia-reperfusion damage.

3. Ionizing radiation is ROS third major source. Cosmic rays which ceaselessly bombard the Earth, radioactive chemicals, and the X-rays are all forms of ionizing radiation (12).

The terms "flavonoids" and "bioflavonoids" are identical. They act as antioxidants. The term designates a class of *secondary plant metabolites* based on the structure of phenyl benzopyron. The synthesis of flavonoids begins with a product of glycolysis – phosphoenolpyruvate, which follows a shikimate pathway to give phenylalanine. The latter is the starting point in the phenylpropanoid metabolic pathway. It is the raw material for 4-coumaryl CoA, which can combine with malonyl-CoA to give the skeleton structure of the flavonoids (a group of compounds, called chalcones). These compounds have a three-ring phenol structure, which explains why they bear the name of polyphenols. The simplest chemical structure within the polyphenol group is the one of the quercetin. The metabolic pathway goes through a number of enzyme modifications until flavanols are obtained. They give the origins of dihydroflavanols, known as **anthocyanins**. A lot of products (polyphenol compounds) can be obtained using the same pathway: flavan-3-ols, proanthocyanidin (tannin), etc. The flavonoids are widely distributed in plants, performing a number of functions, the major one being to produce yellow, red, or dark violet pigmentation in flowers and fruits and to protect against the attacks of microbes and insects. Research shows that when taken in food by people, flavonoids demonstrate a stronger antioxidant effects than the classic-type antioxidants – vitamins C, E, and β-carotene, and this is due to the following mechanisms: 1. They prevent free radicals formation by chelating the heavy metal ions participating in Fenton's reaction. 2. They catch the free radicals and form less active compounds which are subjected to reduction. 3. They catalyze the neutralization process of free radicals by way of the natural antioxidant enzyme systems of the cell till neutral products are derived. Flavonoids also inhibit the activity of the enzymes involved in the arachidonic acid cascade –lipooxygenase, cyclooxygenase, phospholipase A_2; they suppress the secretion of lysosome enzymes and histamines from the adipocytes, which determines their anti-inflammatory effect, too (2).

The physiologically justified lifestyle (diet pattern and optimized physical exercise) recommended by Barry Sears in his "Zone" aims at overcoming the insulin resistance (1). Alongside, there is enough evidence of decreased free fatty acids infiltration into the liver, lowered level of pro-inflammatory cytokines – α tumor-necrotizing factor (TNFα), visfatin, interleukin 6 (IL6), interleukin 8 (IL8), resistin, as well as of increased level of anti-inflammatory cytokines, such as adiponectin. It has been established that high levels of adiponectin have a protective effect against NAFLD in mice (5,15). This is explained by inhibiting the expression and the activity

of acyl-CoA-carboxylase and the synthesis of fatty acids. "The In-Zone Stay" which our patients will adopt as their lifestyle for long years in the future leads to a favourable change in all correctable factors of the cardiovascular risk, respectively, on the metabolic syndrome (1).

The authors of this study have applied the most frequently used and easily accessible diagnostic methods for NAFLD-steatosis in the country. Thus, employing clinical criteria we can presume, but we cannot doubtlessly ascertain the NAFLD type, which impacts on the prognosis. There exist other kinds of combined medical investigations which enable to identify with a high degree of certainty the extent and localization of fibrous changes in the liver making application of non-invasive methods (without histologic analysis). Examples of these are FibroTest/Metavir, FibroScan, ActiTest, SteatoTest, NoshTest, AchTest, and others. (7). To acquire even more reliable conclusive evidence the methods are used in combination of two. In these cases, the laboratory panel is a must, and when investigating for steatosis it includes: thrombocytes, ASAT, ALAT, GGT, ferritin, hyaluronic acid, prothrombin time. The total results are validated by an expert computer system (11).

The new approach of keeping a healthy lifestyle, called "*Phenotypic plasticity*" defines the role of diet and physical activity and the way they impact the ageing process, as well as the importance of phenotype plasticity as a potential biomarker of the good health state. "You are what you eat and what you have already eaten, and the information about this has been delivered, recorded, and saved into your genome". These words were said by Prof. Michael Muller from Wageningen University (Holland), who dealt on the mechanisms of metabolic/phenotypic plasticity that sustain the lipids homeostasis in the body. The lipids and the other nutritious substances, which modulate genetic expression by way of nucleus receptors and changing chromatin activity have a major role in the manifestation of metabolic plasticity, for instance, the plasticity of the digestive tract. Prof. José Viña of University of Valencia presented the process of ageing as a process of losing metabolic plasticity and disrupting the homeostasis. Longevity has been associated to changes in gene expression that are similar in a hundred-year-olds and adolescents. The theory of the role played by active oxygen forms (AOF) as participants in the signal transduction, the production of which is crucial for keeping a person's good health and contributing to longevity, was elucidated by Prof. Michael Ristow from ETH, Zurich. He presented the view of mitohormesis as a general mechanism contributing to the physiological adaptation. The AOF are the actual intermediaries in the adaptation mechanisms. Physical exercises, reduced calories intake, limited carbohydrates intake are all factors determining the metabolic plasticity conditioned by ***mitohormesis.***

Our research met its goal. We offered non-medication treatment to patients who have been diagnosed with NAFLD by routine methods, without biopsy. Each of them passed a thorough course on how to cope with life in the "Zone". Our previous studies on the activity of anthocyanins suggested that they should also have hepatoprotective effects. This has been proved experimentally on rats which had their liver damaged by CCL4. The experimental animals had their liver histologically investigated before and after they were give aronia juice. Significant improvements in histological results have been reported with regard to necrosis, balloon degeneration, and inflammatory infiltration of lymphocytes (15). Similar favourable effects have been registered by other authors who used various liver damaging agent: aminopirin and sodium nitrate, cadmium chloride (2, 9). Apart from histological changes, there is data on normalizing the serum levels of bilirubin, urea, and aminotransferases.

Literature offers no suggestions on the therapeutic dosage of aronia melanocarpa juice intake for humans. Some communications recommend that the dose should be conditioned by kg/weight relation, others take age as an indicator (14). The dose we used can be recommended as an optimal one for this type of application. One should consider the fact that the dose depends on the juice quality. The juice depends on the quality of fruits and the contents of anthocyanins in them, for the specific year (3). For protective purposes it will suffice to take 50 ml of juice daily, 4 times a week. The therapeutic dose is 200 ml juice of aronia melanocarpa a day. On finishing the research we advised our patients to continue the juice intake for another month at a dose of 100 ml/day divided in 2-3 portions. The dose recommended is only indicative. It can always vary by±15 ml.

Not a single respondent to our enquiry has indicated any prior information or knowledge of aronia fruits or Barry Sears system. The changes that have taken place in Bulgaria's health policies and the recent emphasis on preventive strategies will hopefully enable changes in this field. The public needs to be given access to more information and training in a bid to overcome their harmful habits and encourage a routine of keeping a healthy diet.

The fruits of aronia melanocarpa are rich in a number of vitamins and microelements (Table 8). Some of the ingredients are valuable as cofactors of the cellular enzyme antioxidant systems. Last, but not least – the product does not contain synthetic substances. The qualities of natural fruits have been preserved. We could recommend such fruits in the daily menu for health prophylactic purposes, and they could be recommended as a must for people with various diseases, especially in the recovalescence stage, following severe therapeutic interventions. Aronia fruits are suitable for people who have been exposed to comparatively high UV radiation. The American FDA (Food and Drug Administration) approaches the special terminology and classifies foods by marking them by a Functional sign (when the food relates to keeping normal functions of a certain structure or system) and by a Health sign (when the food relates to„diet-disease"). It is necessary to distinguish between "functional food" and "food supplements". The latter have become an economic hit, but the benefits they bring can be considered negligible, and the harmful action – much more frequent. FDA has given a Health sign only to 11 functional foods over the last year. In our humble opinion, fruits of aronia melanocarpa may be included in the classification as functional food with a health sign.

Conclusion.

The independent pathogenic role that MS plays evolves against the background of a continually developing risky factors where sedentary way of life, overeating, and stress supplemented by harmful environmental influences, such as smoking and ionizing radiation keep on building up conditions for the syndrome progress. The importance of this problem is to be found in the fact that it is socially significant since 6-7% of young people (aged 20-29) and about 40% of people above the age of 60 suffer from it. It is therefore important to educate large circles of population how to maintain a healthy lifestyle, which is elucidated and motivated in the "Zone". From the way of life, through hormone mechanisms of metabolisms and the action of a series of cytokines we got to the oxidative stress of cells – which is the specific manifestation of the tissue damage. The complex of a health-promoting lifestyle and the intake of natural foods with antioxidant effects is the intelligent choice of the present-day working person. The authors of this study attained its goal

– to establish in a scientifically justified manner the beneficial effects of the intake of aronia juice. NAFLD-steatosis patients who have passed the training described in Barry Sears' "Zone" lifestyle and who persist in observing its requirements coupling them with 200 ml/day aronia melanocarpa juice in two months' therapeutic course reach laboratory values within reference range for their liver function parameters, and the metabolism carbohydrates and fats. At control sonography in the end of the second month, the same evidence regarding steatosis findings was negatively confirmed in the ultrasound image. On comparison with patients who apply the "Zone" principles of life but were not given aronia melanocarpa juice, subjects incorporated in the study demonstrated significantly higher speed of recovering their optimal metabolism. Another major advantage worth mentioning is that all who have taken aronia melanocarpa juice report favourable effects on discomfort of varying nature they have lived with for a prolonged period preceding the research and the newly acquired feeling of general wellness.

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