CHAPTER 10

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MODERN METHODS OF NON-INVASIVE ASSESSMENT OF THE STATE OF PROVISION OF PARTICULAR NUTRIENTS

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Abstract: *An analysis of recent studies on non-invasive technologies for assessing the state of provision of particular nutrients as key factors of human health and physical development is provided. The peculiarities of determining the nutritional status, the role of environmental factors, and the features of assessment in conditions of nutrients emitting toxicity are being given. While generalizing various approaches to the analysis of the provision of particular nutrients, several conclusions regarding the prospects for the further development of non-invasive assessment was given. These prospects include a new complex-systemic approach based on laboratory studies, application of standard clinical assessment schemes and functional tests. They also include the assessment of environmental factors and instrumental methods for assessing actual nutrition, designed to answer the results of the latest scientific research and to increase the efficiency of determining determining the nutritional status in order to achieve effective prevention of non-communicable diseases and prolong longevity.*

Keywords: *Non-invasive assessment, Ecosystems, Nutritional status, Nutrients, Methods, Green economy.*

INTRODUCTION

The assessment of the state of provision of particular nutrients is used in medical ecology, food hygiene, cosmetology, fitness, dietetics, sport-sanitary medicine and clinical practice, which allows reducing the incidence of complications, accelerating recovery or the onset of remission, and increasing the effectiveness of pharmacotherapy by adjusting nutrition, which is of great socio-economic and medical importance (Reber, 2019; Bashun et al., 2020). It is known that the nutritional status is considered as a certain state of health and physical development, the physiological state of the body, formed under the influence of the quantity and quality of nutrition, taking into account one's needs for nutrients and energy, and genetically determined and acquired individual characteristics of the nutritional metabolism, as well as the state of organs and systems involved in digestion, absorption and excretion of nutrients (Ragin et al., 2019; Bashun et al., 2017a).

Depending on the results of the assessment, nutritional status is usually divided into optimal or adequate (McGuire, Beerman, 2017), normal, overnutrition and undernutrition (Bashun et al., 2020). Optimal nutritional status is an important factor for health and well-being, and it should be achieved by adequate intake of dietary energy, essential nutrients, and other food components (e.g. fiber), and by absence of toxins and contaminants in food (McGuire, Beerman, 2017; Nutrition Assessment, Counseling, and Support (NACS), 2016). The optimal status for a particular nutrient between its shortage and excess determined by functional assessment is also considered. Normal nutritional status is not strictly defined. This collocation is usually used to characterize the state of provision in clinical conditions or in case of diseases. This status may deviate in any direction in some components from the optimal one (Reber, 2019; Zhou, 2015). Overnutrition occurs as a consequence of excess nutrient and energy in relation to the energy consumption (Ragin et al., 2019; United Nations Children’s Fund (UNICEF) et al., 2019). Commonly distinguished types of overnutrition are overweight and obesity (FAO et al., 2019), as well as an excess of a particular nutrient sometimes, which can lead to specific health problems (chronic diseases) or (acute) toxic effects (McGuire, Beerman, 2017). Given the fact that it is difficult to accurately define or assess optimal health status, more attention has (traditionally) been given to different forms of undernutrition. Undernutrition, or malnutrition, is formed due to quantitative and especially due to qualitative in adequacy of nutrients and nutritional energy, in conditions of depletion of their internal reserves and compensatory metabolic pathways (Bashun et al., 2020). Undernutrition can be subdivided on the basis of a number of criteria, including anthropometry data, dynamics of growth and development, its etiology and pathogenesis, influence on development and function, severity (Gandaeva, 2015). Some examples of different types of undernutrition are wasting, thinness, child stunting, vitamin deficiencies, mineral deficiencies (FAO et al., 2019), as well as pathological conditions and diseases caused by them (anemia, scurvy, goiter, etc.) and etc. (Gandaeva, 2015).

Nutritional status can be assessed both at individual and population levels (Baranovsky, 2017). Both overnutrition and undernutrition are considered malnutrition (Ragin et al., 2019), (the term "sub-optimal nutritional status" is also often used) (FAO et al., 2019). Such terms as *eutrophy* (*normotrophy*), *dystrophy* (*hypotrophy and paratrophy*) are also used in pediatrics to characterize the nutritional status of young children. According to the data published by FAO, IFAD, UNICEF, WFP and WHO in 2019, *the number of cases of obesity continues to rise* in all regions of the world and in all age groups, especially among schoolchildren and adults. As for 2018, the number of overweight children under the age of five was estimated at 40 million; as for 2016, 2 billion adults, 207 million teenagers (10–19 years old) and 131 million schoolchildren (5–9 years old) were overweight. At the same time, about a third of overweight teenagers and adults and 44% of children aged 5–9 years were obese. The number of cases of overweight and obesity is rising in almost every country. At the same time, more than 2 billion people, including 8% of the population of North America and Europe, lack regular access to safe, nutritious and sufficient food. Despite the fact that one of the 17 Sustainable Development Goals proclaimed by the United Nations in 2015 is the complete elimination of hunger around the world by 2030, the tendency of a slow decrease in the number of hungry people with chronic undernourishment, which lasted for decades, reversed in 2015. The number of hungry people has been slowly increasing for several years in a row, and it is estimated at more than 820 million people as for 2018. This poses a huge challenge to being able to achieve the Zero Hunger target by 2030, which includes ensuring access to safe, nutritious and sufficient food all year round and *eliminating all forms* of malnutrition (FAO et al., 2019).

All existing methods used to assess the state of supply of particular nutrients are more or less indirect, based on the non-absolute degree of correlation between the determined indicators and nutritional status (Bashun et al., 2020). Therefore, several (groups of) methods at once (integrated assessment) are generally used to assess the nutritional status (Ragin et al., 2019; McGuire, Beerman, 2017). Several groups of methods are usually distinguished, such as anthropometry, methods for determining the composition of the body, laboratory research, clinical assessment, diet analysis and functional tests (Ragin et al., 2019). Also, the assessment of environmental factors (elements of the natural and anthropogenic environment, including the technogenicone) is often used. Among such factors are demographic factors, assessment of socio-economic status (McGuire, Beerman, 2017; Igbokwe, 2017), including certain (groups of) products being banned due to religious reasons, the level of education

(Bashun et al., 2019), access to drinking water and sanitation, etc. Sometimes, these are also combined by the term "ecological factors" (in terms of the ecosystem).

Non-invasive techniques are now widely used to assess the state of provision of particular nutrients. One often gets the research results in a few minutes, which is generally faster than using time-consuming invasive techniques, among other advantages, such as convenience, greater safety and possibility of using those outside the clinic (Ragin et al., 2019). It is known that a non-invasive examination method is a method that doesn't violate the integrity of the skin.

The focus of this chapter is the latest research on methods of non-invasive assessment of the state of provision of particular nutrients, methods for studying nutritional status, as well as our own experimental data. The study of the correspondence of the provision of particular nutrients to the needs of the body, the detection and timely correction of malnutrition that increases the risk of developing health disorders is an important part of the prevention of non-communicable diseases (Bashun et al., 2020; Bashun et al., 2017a), a way to reduce mortality, increase overall well-being, prolong painless period of life, ensure longevity and preserve human capital.

LABORATORY STUDIES

Nowadays laboratory (biochemical) methods allow for a non-invasive assessment of the **state of provision of particular nutrients** (Reber, 2019; McGuire, Beerman, 2017). Out of the non-invasive laboratory methods for assessing the nutritional status of particular nutrients, based on biochemical indicators, urine analysis is widely used. The modern version of it uses automatic biochemical analyzers (e.g. Flexor XL), which makes it possible to detect the deficiency of water-soluble vitamins, macro- and microelements, protein (Bharadwaj, 2016; Bashun et al., 2017d; Bashun et al., 2017b). For example, the provision of calcium is determined by the calcium-creatinine ratio (CCR) in the second portion of morning urine (Bashun et al., 2020; Bashun et al., 2017c). Such methods are used, in particular, in Belarus and Ukraine (Ragin et al., 2019; Anufrik et al., 2017). The content of elements required in nutrition is determined, for example, with the use of atomic absorption spectrometry (Bashun et al., 2020) or X-ray fluorescent (XRF) analysis. An innovative laboratory method for assessing nutritional status in relation to essential elements called elemental hair analysis is already available as a paid service (Anufrik et al., 2017). The disadvantage of this method lies in the influence on the results of the analysis by domestic hair treatment and external pollutants. The reference values of the content of chemical elements in the hair, according to the 2013 systematic review, vary significantly, so the conclusions of various research and commercial laboratories are often incomparable and even contradictory.

Nails can also be used instead of hair for elemental analysis. The advantage of studying hair and nails is the ability to identify biomarkers that characterize food intake over several months or years. For example, nail analysis can characterize the level of selenium consumption over six to twelve months (Bashun et al., 2020).

It is theoretically possible to assess not only the state of provision for particular nutrients, but also the nutritional status in general, including the development of reference values for the purpose of introducing laboratory assessment of nutritional status into practice, based on hair analysis. It could find use, incl. in clinical practice. Mention should be made of some recent studies in this field. So, in 2009, a statistically significant ($p < 0.05$) positive correlation was found between the body mass index (BMI) and the level of nutrients Na, K, Cr and the toxic element Cd in the hair of adult individuals. At the same time, other studies show no correlation between the concentration of Cr in the hair and obesity, or even a negative correlation between this element and overweight plus obesity, in comparison with normal body weight. Taken together, these data do not allow the use of this element as a marker of general nutritional status and require further research.

In the hair of adults a significant correlation ($p < 0.05$) was also established between the nutritional characteristics in terms of the energy and protein components and the level of Mo and Mn in the hair, respectively. In 2004, for 3–6 years old children, a statistically significant positive correlation ($r = 0.159$) was shown between the level of Zn in the hair and body weight, but no such correlation was found with the consumption of Zn in the diet. Another study shows, on the contrary, that the level of Zn in the hair of obese individuals is significantly lower than one in the hair of non-obese subjects ($p < 0.001$) (Sharmanov et al., 2018), which also obviously does not allow relying on hair elemental analysis in this regard, at least as a sufficiently universal criterion suitable for different populations (and groups).

Thus, the assessment of the state of provision for particular micro- or macronutrients remains among the generally unsolved problems in the context of non-invasive laboratory methods and leaves room for the development of methods for such an assessment (McGuire, Beerman, 2017). While using them, however, one can also assess the state of provision for particular nutrients if the corresponding nutrient, its metabolite(s), and/or substances associated with this nutrient through some metabolic pathways become a biomarker of nutritional status. It seems that the development of economically viable screening methods for assessing the nutritional status of the population, based on the determination of biomarkers of nutritional status in biological fluids and/or other biomaterials obtained in a non-invasive way, is possible. Currently, biomarkers from blood plasma are used to assess nutritional status in the laboratory, e.g., as noted, serum albumin, total leukocyte count, etc., which requires an invasive procedure for obtaining biomaterials (Bashun et al., 2017d). An issue requiring detailed development is also the selection of the most relevant biomarkers that would have a high degree of correlation in relation to nutritional status in general, or at least nutritional status in terms of macronutrients and/or many micronutrients; the confirmation that this correlation exists, the assessment of its nature (value), and its sufficiency for use in the basis of the new assessment method in practice are of great importance too.

The study of 2018 as a useful way of development of this direction. This study compares the diet of children aged 9-10 with normal weight and the diet of overweight children (boys and girls separately). It shows that the diet of the overweight boys contains more calories and nutrients (proteins, fat and carbohydrates) as well as micronutrients (calcium, magnesium, iron, vitamins A, D, thiamine, riboflavin, niacin, pyridoxine, folate, pantothenic acid, biotin, vitamin C) than that of the normotrophic boys. The difference is statistically significant: according to the Student's t-test, $p < 0.05$. One can use prospectively each of these nutrientsrespectively as a marker of nutritional status (distinguishing between overnutrition and undernutrition), especially when it comes to the nutrients with the statistical significance of the difference $p < 0.01$: iron, thiamine, riboflavin, niacin, pyridoxine, vitamin C. Out of them, those potential laboratory biomarkers of nutritional status are the best for which a statistically significant correlation $p < 0.01$ remained for girls: iron, thiamine, niacin, pyridoxine. Thus, the assessment of biochemical parameters is part of the basic assessment of nutritional status in the ABCD approach, along with anthropometry (A), diet assessment (D) and clinical parameters (C) (Nutrition Assessment, Counseling, and Support (NACS), 2016).

CLINICAL ASSESSMENT

Examination of a patient (physical examination, somatoscopy) allows a specialist (a doctor, in particular) to diagnose the state of malnutrition and determine the deficiency of certain nutrients, based on the signs of the deficiency, but only in manifest forms. At the same time, these symptoms and signs are often nonspecific, which reduces the reliability of the method. Therefore, it is necessary to supplement the clinical assessment with laboratory tests (Bharadwaj, 2016). Clinical assessment can replace anthropometry or determination of biochemical parameters of adults, but is insufficient for children in comparison to anthropometry. Signs of nutritional deficiency, including a deficiency of certain vitamins and microelements, are determined on the basis of a subjective examination, or a collection of anamneses (patient's clinical history), which makes it possible to assess the state of internal organs, functions of the musculoskeletal, immune, and nervous systems, etc., and, further, on the basis of examination data or physical examination. Characteristic signs of undernutrition (including lacking macro- and micronutrients), in particular, are associated with tissues with a high rate of cellular renewal. Chronic malnutrition is marked by visible changes of other tissues. A doctor can assess these changes, such as peeling skin, chapped lips, erosion of the oral mucosa, pallor of the skin (as a sign of anemia), protrusion of the bones of the skeleton, depigmentation of the skin and loss of its elasticity, thin, rare, easily pulled hair and their depigmentation, muscle weakness, decreased mental and physical performance, etc. (Reber, 2019). Deficiency of certain vitamins and microelements is manifested by characteristic symptoms corresponding to the deficiency of the particular micronutrient (Reber, 2019). For example, bleeding of the gums is a sign of vitamin C deficiency. Same applies to macronutrients: for example, the presence of general edema may indicate a lack of protein in food (McGuire, Beerman, 2017). One can also identify the signs of the excess of certain nutrients: for example, loss of appetite is a sign of zinc intoxication (McGuire, Beerman, 2017).

The most recent options for clinical assessment of nutritional status include the use of standard schemes. The first such scheme, called the Subjective Global Assessment (**SGA**), was introduced in 1987 (Reber, 2019; Bashun et al., 2020). It is widespread in the world clinical practice. This scheme is recommended for detecting nutritional status disorders or the risk of such a disorder during hospital stay. SGA is a simple and inexpensive technique. Its criteria for diagnosing undernutrition include food intake, weight loss, gastrointestinal symptoms, level of physical activity, body temperature during previous 72 hours, atrophy of subcutaneous fat and muscle tissue, and the presence of general edema or ascites (Bashun et al., 2020). The degree of malnutrition according to the SGA criteria is significantly correlated with such indicators as the percentage of body weight loss, BMI, the thickness of the skin-fat fold above the triceps, and the level of serum albumin and prealbumin. A number of later developed clinical schemes, in contrast to SGA, may include data from invasive studies (for example, determination of the level of serum albumin) as diagnostic criteria. Such schemes are beyond the topic of discussion. Many other schemes, developed after SGA, are used for screening (Zhou, 2015), which is a faster assessment of the patient's nutritional status, with the possibility of referring to the SGA for a more detailed assessment in case of suspected nutritional disorders, according to the screening data (Reber, 2019; Bashun et al., 2020). Thus, the Malnutrition Universal Screening Tool (**MUST**), recommended by the European Society for Clinical Nutrition and Metabolism (ESPEN) (Reber, 2019), was developed relatively recently for widespread use by the British Association for Parenteral and Enteral Nutrition (BAPEN). MUST is intended for adult patients and can be used in any medical institution to determine protein-energy malnutrition and the risk of its development through three independent criteria: BMI, unplanned weight loss and the presence of severe disease that led to a decrease in food intake for ≥ 5 days (Reber, 2019). This method is well consistent with the dietary assessment of malnutrition (Bashun et al., 2020).

NRS 2002 (Nutritional Risk Screening 2002) is an algorithm developed based on MUST. This scheme excludes the calculation of the percentage of weight loss (which may be difficult for employed medical personnel) (Reber, 2019). The NRS 2002 uses BMI data, weight loss in the last 3 months, a decrease in food intake in the last week, and the presence of severe illnesses (Reber, 2019; Zhou, 2015). NRS 2002 is also recommended by ESPEN (Reber, 2019); the method has been evaluated and validated in hundreds of studies, including randomized controlled trials, and has been shown to be highly reliable when used by trained personnel (Reber, 2019).

ESPEN also recommends **MNA** (Mini nutritional assessment) – a non-invasive clinical scheme used both for screening of particular nutrient provision and for routine assessment of nutritional status (due to the presence of two parts) (Reber, 2019). Assessment by this method takes less than 10 minutes (Ragin et al., 2019). The shortened version of MNA, Mini Nutritional Assessment-Short Form (MNA-SF), developed later, is designed specifically for people over 60 years old (Ragin et al., 2019; Zhou, 2015).

DIETARY ASSESSMENT (ACTUAL NUTRITION ANALYSIS)

Actual nutrition analysis, or dietary assessment, is widely used to determine nutritional status at the individual and family level – for example, in the well-known integrated nutritional assessment scheme ABCD, along with anthropometry (A), biochemical studies (B) and clinical assessment (C) (McGuire, Beerman, 2017; Nutrition Assessment, Counseling, and Support (NACS), 2016). When analyzing the diet, the consumption of specific foods and / or the nutrients they contain and supplements (tea, etc.) are determined and general patterns of food intake (including frequency and amount of intake), dietary diversity and specific dietary preferences are analyzed (Bashun et al., 2016; Magrini, 2017).

There are retrospective and prospective types of dietary assessment used to assess past and current nutrition respectively (Ragin et al., 2019; McGuire, Beerman, 2017). The two main methods of retrospective nutritional assessment are the 24-hour playback method and the consumption frequency questionnaire, which are varieties of the questionnaire survey method (McGuire, Beerman, 2017). It also includes the method of usual consumption (Bashun et al., 2016; FAO, 2018); the method of analysis of menu-plan is also used for the retrospective assessment. The methods of prospective assessment include keeping a food diary (Archundia Herrera, Chan, 2018), the food record method (Ragin et al., 2019; McGuire, Beerman, 2017), direct observation, the duplicate portions study method, etc. Recently, in addition to this, using data automatically collected by electronic devices designed for self-dependent monitoring of nutrition for prospective assessment has become possible (Archundia Herrera, Chan, 2018; FAO, 2018).

Obtained nutritional data are subsequently analyzed with the use of tables of the chemical composition of food products. There's a newer way to do so involving easy-to-use computer programs (Magrini, 2017), which include databases (providing information on content of nutrients and energy in many types of food) (McGuire, Beerman, 2017; Bashun et al., 2016; FoodDataCentral, 2020). Examples of such databases are Food Data Central (US Department of Agriculture, USDA), which features enhanced nutrient profile data and links to relevant agricultural and experimental studies (Information and analytical system, 2020); and "Chemical composition of food products used in the Russian Federation" — a database in Russian (Diet History Questionnaire III, 2020). The analysis of the questionnaires is performed manually (Information and analytical system, 2020; Diet History Questionnaire III, 2020) or automatically (calculating the nutrients based on the results of the questionnaire). The obtained data can be used for further comparison, for example, with the official norms of physiological requirements for energy and nutrients for different groups of the population, depending on the nature of work, age and functional state of the body. These norms approximately correspond to the reference values of consumption (Dietary Reference Intake (DRI) Standards), officially adopted in the United States and Canada and including Estimated Average Requirements (EARs), which characterize the average nutrient requirements for a population, and the *recommended intake of a nutrient* (Recommended Dietary Allowances, RDAs) at the individual level (McGuire, Beerman, 2017). When it is difficult to establish the EAR and accurately determine the RDA as a consequence of this, as for example, in case of pantothenic acid (McGuire, Beerman, 2017), one should use the Adequate Intake (AI) Level (McGuire, Beerman, 2017). As the fourth component of DRI we use the Upper Tolerable Level (UTL, UL) to assess excess dietary intake and toxicity (McGuire, Beerman, 2017).

The analysis of actual nutrition is carried out with the **24-hour recall** methods: patient is to recall all the food and liquid intake during previous 24 hours (McGuire, Beerman, 2017; Bashun et al., 2016; FoodDataCentral, 2020). This method is widely used in the world practice of dietary assessment of nutritional status. A modification of the method is the **interactive 24-hour recall**, designed specifically to collect information from people living in the rural areas of developing countries. In order to improve the reproduction of food consumption, the researcher can set up group training on determining portion sizes before the questionnaire; provide the examinee with a set of images of food the day before the interview to compare these images with the consumed food and use the set during the interview; provide utensils to facilitate visualization of portion sizes during memory playback; use weighing of duplicate portions. Another modified version of the 24-hour recall technique was suggested in 2016. It is called ***current-day dietary recall*** (***current-day recall***) (Bejar, Sharp, García-Perea, 2016). This method is (initially) based on a smartphone app called electronic *12-hour dietary recall* (e-12HR) (Béjar L.M. et al., 2019). In the context of the assessment of usual consumption, the **method of usual food intake** or food anamnesis is also used: the subject is asked to list from memory the range of food usually consumed at a particular time of the day (Bashun et al., 2016). **Food frequency questionnaires** (FFQs), or food frequency analysis method, are questionnaires containing a list of questions about the frequency (and size) of specific foods and food types over a period of time, usually per day, week, or month (McGuire, Beerman, 2017; FoodDataCentral, 2020). **Keeping a food diary** (diet record) **without weighing food** (estimated food diary, estimated-diet record) or a simple food diary, or a method of estimating the amount of food consumed by the subject, is that the subject (or a parent or other family member or a caregiver) is instructed to keep records, directly during meals - of all food and drinks, for a certain period of time, usually 3-4 days, with the obligatory proportional inclusion of weekends (McGuire, Beerman, 2017). The **food account method** implies, in contrast to the food diary, keeping records not of food eaten, but *of all purchased or received products* - in trade units or household measures of weight / volume; the record is kept by the head of household or the person responsible for purchasing most of the products, usually within a week. At the population level, nutritional assessment is also possible using data from ***retail chains***. **The weighed food record method** is usually considered *the most accurate method for assessing the usual intake of food and nutrients* at an individual level (FoodDataCentral, 2020). **The duplicate portions study method** is used to assess nutrition for macro- and micronutrients, and also allows to identify toxic substances (heavy metals, pesticides) and contaminants in food. For example, the method was used in the Netherlands by the National Institute of Public Health and Environmental Protection, based on a random sample of the population aged 18–74 living in the Utrecht region (Ochuizen et al., 1991). Assessment of usual consumption at the individual level, based on data for a relatively long period of time (usually a month; also - less than a month, and no more than a year) is a **dietary historymethod** (FoodDataCentral, 2020); in its original form (proposed by Burke, 1947), the method includes three components. The first one is an interview about the usual food intake during the day, the second a frequency of consumption questionnaire (at this stage, 24-hourly playback) and the third keeping a simple food diary for 3 days. Nowadays, the third component is rarely used (FoodDataCentral, 2020). As noted, in recent years, there has been an active development of the field of research on the possibility of using **electronic devices** (mainly portable) designed for the ***automatic collection and processing of dietary data*** for dietary assessment. Together with the use of photographs as part of dietary assessment, these can be considered as a new group of methods: innovative methods of dietary assessment, in addition to prospective and retrospective assessment methods (FoodDataCentral, 2020).

In general, dietary assessment remains an important and available direction in the arsenal of specialists who assess both the nutritional status in general and the state of provision of individual nutrients. It provides a wide range of methods to choose depending on the objectives of the study, the studied group of respondents, etc.; many methods have been tried and tested for decades, and the latest electronic devices open up prospects for the further development of dietary assessment, improving its convenience and accuracy.

ASSESSMENT OF THE ENVIRONMENTAL FACTORS

Recently, increasing attention has been paid to the influence of the environment on nutritional status (Bashun et al., 2020). One can find a large, unlimited set of environmental factors, also called, as noted, ecological. This factors can affect the state of nutritional provision: family composition, level of education and income, drinking water supply and sanitary conditions, access to health care and agriculture, food prices, cooking conditions, exposure to advertising that changes eating behavior and others (FAO et al., 2019).

Particular factors are of more or less significance depending on the region of residence (e.g. extreme climatic events, conflicts (FAO et al., 2019), etc.). Some factors are quite universal. These factors are analyzed more often than others and are often distinguished into separate groups of methods, for example, assessment of demographic factors and determination of economic status, and socio-demographic and socio-economic assessment, which are close or synonymous to them, respectively (Igbokwe, 2017). Sometimes, more specific factors are distinguished into separate methods or directions: for example, family assessment (a household that can also consist of 1 person), within the framework of a variant of the ABCDEF method, where F means Family / household assessment (Bashun et al., 2016). It should be noted that the analysis of diet (actual nutrition) is usually not considered as part of environmental factors.

The noted methods (directions) can be used as part of the *assessment of the impact of environmental factors* on the green economy (FAO et al., 2019), as well as separately: for example, by assessing the economic status (E), sometimes they expand the noted scheme (complex of methods) for assessing the state of provision ABCD (McGuire, Beerman, 2017). **Assessment of demographic factors** is sometimes used in combination with other methods (approaches) to determine nutritional status. Among demographic factors, the state of provision with macro- and micronutrients can be significantly influenced by the *region of residence* (Berkhout et al., 2019). Deficiencies of such microelements as selenium and some others, as well as some vitamins (for example, vitamin D in countries with relatively low insolation for its synthesis in sufficient quantities in the skin), are also widespread, depending on the region. Thus, living in such regions is associated with an increased risk of developing deficiency according to these micronutrients and requires measures to prevent the development of their deficiency among the population (for example, in Belarus salt is iodinated; and in some EU countries vitamin D and folic acid is added into flour, spreads, milk spills, etc.). In 2019, a statistically significant association of micronutrient composition of soils with the prevalence of stunting, underweight and wasting among children was found in sub-Saharan Africa due to theuse of new maps of soil composition for micronutrients (Arjuna, 2017). Therefore, it can be concluded that the assessment of the mineral composition of soils can serve as a new, alternative method for predicting and determining malnutrition at the population level, of course, for particular regions. *Family size* may be another demographic influencing nutritional status (FAO et al., 2019). So, in some regions (for example, West Africa), in large families (more than 7 children), on average, a higher risk of the formation of insufficient nutritional status is observed. Among the demographic factors that affect nutritional status are *gender, age, ethnicity, and living in urban or rural areas* (FAO et al., 2019). Thus, in the population of the southwestern region of Nigeria, there is a negative, statistically significant ($P<0.05$), correlation of age and BMI (Igbokwe, 2017). The nutritional status of the rural population is on average lower than that of the urban. *High population density* can also be the cause of inadequate nutritional status (malnutrition). It is widely believed that on a global scale, continued population growth is a factor that increases the risk of an inadequate nutritional status on average for the world's inhabitants, and may be a threat to food provision and public health in the near future. At the same time, the first scientific approach considering population growth as a problem due to limited resources and the possibility of catastrophic consequences was created by *T. Malthus* in the 18th century. According to this model, the growth of the human population can be exponential, similar to the growth of a bacterial colony on a nutrient medium in the absence of limiting factors, until the medium is depleted; Malthus's exponential law, in the context of ecology, is currently being compared with Newton's First Law in physics. Malthus criticized the concept of J. Condorcet in a treatise of 1798, considering the growth of the human population as a negative factor leading to the growth to poverty and vice and, ultimately, decline in population due to the limited resources, especially those associated with food production, because of their slower, non-exponential growth, lagging behind population growth.

As noted, demographic factors are closely related to economic factors also used in assessing nutritional status and can determine the latter: for example, the income level in urban areas in some populations is on average higher than in rural areas. According to Malthus, population growth prevents the increase of per capita income or makes it temporary only due to the introduction of the scientific discoveries and technological progress, leading to a more dense population while the provision (of food specifically) stays the same, as shown in his work (for the period 1–1500 AD) and for the period of 100,000 years of the preindustrial era (before 1800). This can be the main cause why the problem of insufficient nutritional status still exists and becomes even worse despite the long path of scientific and technological progress. In other words, this explains why the problem is still relevant and growing.

Economic factors, such as income levels and food prices, influence consumers' behavior to food consumption and, as a consequence, determine the emerging of nutritional status.

It should be noted that both over- and undernutrition lead to economic costs and a slowdown in human development (Parkanskaya, 2016), creating a vicious circle. At the same time, the economic losses in the world due to the prevalence of overweight and obesity are enormous and estimated at $2 trillion per year (FAO, IFAD, UNICEF, WFP and WHO, 2019). Undernutrition according to forecast will reduce GDP in Asia and Africa by 11%. In early 2019, the IMF revised its global growth forecast to its lowest level since the global financial crisis a decade ago. In general, the state of the global economy is pessimistic and the latest forecasts for the future are alarming (FAO et al., 2019). Economic assessment can be recommended as a very informative criterion in relation to nutritional status. It can be used for screening, as an additional question (about income), while visiting a doctor, while using a questionnaire method for assessing actual nutrition, or as an addition to clinical assessment schemes, taking into account psychological and other factors.

FUNCTIONAL TESTS

Functional indicators are used to assess the state of provision of particular nutrients and nutritional status in general. In some interpretations of the scheme (set of methods) ABCDEF, functional assessment is included under the letter F (Functional assessment) (Baranovsky, 2017). Functional tests, as noted, can be considered as a part of laboratory methods for assessing nutritional status. There are functional physiological tests, behavioral tests, and functional biochemical tests.

Functional biochemical tests assess the *activity of enzymes*, which depends on the level of the nutrient, in particular, by the presence of **atypical metabolic products** in the biological fluid (urine), due to the reduced activity of the enzyme (xanthurenic acid with a lack of vitamin B6, forminoglutamic acid - with folate deficiency, methylmalonic - in case of vitamin B12 deficiency). Also, functional biochemical tests include some **samples with loads of substances**. It is a variety of load tests, which are widely used in assessing the *state of provision of particular nutrients*: in the diagnosis of deficiencies of water-soluble vitamins and some microelements (for example, Mg, Zn, Se). There are several examples of these tests: a test with a load of the amino acid tryptophan is used to assess the state of provision with pyridoxine and test with a histidine load is used to assess the level of folic acid (folate) in the body. The method is based on the nutrient deficiency in a decrease in the activity of some enzyme(s), for which the studied nutrient (or its metabolite) is a cofactor or a prosthetic group, and in changes in metabolic pathways, which can be assessed through load tests with appropriate substances, which allows the latter to be used as indicators of the nutritional status of the nutrient under study.

Functional physiological tests include (among the most well-known and accessible ones) assessment of the function of night (twilight) vision, *dark adaptation test* as an indicator of vitamin A and B2 supply, *study of the pungency of taste* as an indicator of the provision of zinc and vitamin A (gustometry method), *skin capillary resistance* to assess vitamin C and routine status.

The dark adaptation test, at the present stage, can be performed, for example, with the use of adaptometer devices. There are two types of such devices. The first type determines the threshold light sensitivity of the eye in absolute numbers, and the second one detects a decrease in light sensitivity indirectly by the time of detection of the Purkyně phenomenon. The latter can also be estimated in a more traditional, approximate (simplified) way using the Kravkov-Purkynětable.

Assessing the pungency of taste is carried out by noting the taste threshold according to the method of drip stimulation (increasing concentration of a substance that stimulates the taste receptor). The threshold of taste sensitivity can also be measured using an electrodegustometer.

Skin capillary resistance (SRC) is assessed by the number of minor petechial hemorrhages on an area of the skin after standard (dosed) mechanical action (excessive or negative pressure).In assessing the provision of vitamin C, this test is not specific to the required extent.

An example of a functional test that allows assessing the state of provision for several essential nutrients at once is the assessment of *cognitive functions* (this function depends on the nutritional status of iodine iron, zinc, vitamin B12, etc. at the same time) (Magrini, 2017). The advantage of functional physiological tests, when compared to biochemical functional ones, is, as a rule, greater ease of carrying out and a more direct connection with the state of health. However, there is a disadvantage, too: there is a low specificity in assessing the state of nutrition for selected nutrients (the results depend on several nutrients at the same time).

To assess the nutritional status in general, or to diagnose protein-energy malnutrition, tests are used to determine the function of the muscular system, and the growth rate (presence or absence of growth retardation) in pediatrics. Dynamometry, tests with squats and with a step, breathing tests (Stange and Gencha), bicycle ergometry, etc. are used to study **exercise tolerance** or physical performance and to determine the *functional reserves of the body*, in order to assess nutritional status (Magrini, 2017). **Hand grip force** (hand dynamometry) is measured with a dynamometer. It is used to assess nutritional status in clinical practice and for the elderly.

Supplementation can be considered the most reliable test for assessing the state of provision of some microelements (for example chromium (Cr) and zinc (Zn)) according to the criterion of improvement or restoration of impaired function after such supplementation. The disadvantage of this technique, however, is that it is time-consuming and unsuitable for population studies.

It is advisable to use double-blind randomized intervention trials in the development of tests for assessing nutritional status (including for individual nutrients) using the analysis of cognitive function. Significant changes in cognitive function between subjects with insufficient nutritional status (in terms of nutrition) and a control group of healthy subjects are documented; or there is an improvement in cognitive function after nutritional intervention. For example, a study in Kenya investigated whether food of animal origin (supplementation with groups of products of animal origin) plays a key role in the optimal development of cognitive (intellectual) function of school-aged children. According to the results of the study, using **Raven's Progressive Matrices**, a significantly higher degree of indicated functional development was shown in children who received meat supplementation than in children in all the other three groups (supplemented with milk, supplemented with sources of additional food energy (calories), and a control group without nutritional intervention).

The results of the study may be partly explained by the increased consumption of meat as a source of iron and a number of other nutrients, with the level of which, as noted, the connection of intellectual function has been established; the ratio of Fe deficiency to the state of cognitive function among children of senior school age was investigated, for example, using **IQ-tests**.

The influence of the state of provision of Cu and Fe (separately) on sleep function (change in its characteristics in a deficient state for the mentioned essential microelements) has been demonstrated, which indicates the applicability of sleep function assessment as a simple non-invasive test in the study of nutritional status for these nutrients, for example, as an addition to other methods.

Assessment of energy consumption associated with physical activity is sometimes used as an independent method for assessing nutritional status in the context of one of the variants of the ABCDEF approach, under the letter E (Exercise or physical activity assessment) (Bashun et al., 2016). To assess the level of physical activity (for the subsequent calculation of total daily energy consumption) within the framework of the method, the **International Physical Activity Questionnaires** (IPAQ) (Bashun et al., 2020) can be used, representing a set of well-developed tools for obtaining comparable data when used in different regions of the world (Bashun et al., 2016). In assessing energy consumption associated with physical activity the most popular modern method is **accelerometry** (Khalatov et al., 2015).

Among the non-invasive **immunological tests** for assessing the nonspecific immunoresistance of the organism, as an indicator of the degree of nutritional adequacy to physiological needs, that is, the nutritional status, one can define the total amount of superficial skin auto-microflora and the presence of transient forms of microorganisms, saliva bactericidal activity (SBA) and salivary lysozyme.

The research of the state of auto-microflora is carried out with the help of microprints, followed by counting the total number of colonies on meat-peptone agar (MPA), and the number of mannite-decomposing strains of staphylococcus (Korostelev's medium is used). The saliva bactericidal activity against E. coli (gram-negative bacteria) and the activity of saliva lysozyme in relation to Micrococcus lisodeicus (gram-positive bacteria) are determined photonephelometrically. The state of immunity can also be indirectly assessed according to the criterion of acute respiratory diseases in those examined over the past year. In general, functional tests are generally accessible (not labor-intensive, inexpensive), sufficiently informative, both in relation to nutritional status in general and the provision of particular nutrients, and can be used independently, and, as noted, as a component of clinical assessment, which ensures their widespread use in the practice of assessing nutritional status.

CONCLUSION

The chapter notes, in the context of the development of technologies for non-invasive assessment of the state of provision of individual nutrients, the advantages and disadvantages of the main methods of such assessment, at the present stage, including a number of promising development paths, new possibilities for determining the nutritional status based on laboratory parameters, the use of standardized clinical assessment schemes, laboratory (biochemical) studies, functional tests, assessment of environmental factors, as well as new instrumental methods for assessing actual nutrition (Bashun et al., 2020).

In conditions of sufficient resources, it is recommended to use a combination of methods and a comprehensive assessment. This approach, first of all, serves as a response to the results of studies of the recent years about a higher risk of obesity of people with low socio-economic status, associated with a lack of nutrition in many key nutrients. Obesity, therefore, should be defined as an insufficient rather than an excessive nutritional state (or excessive only in terms of the energy component, as it has been interpreted recently). According to Liebig's law of limiting factors (optimum, or optimum-pessimum), the state of an ecosystem is determined by a substance that is in short supply; therefore, when considering the body as a system (as a whole), it should be stated that the lack of even one essential nutrient makes the nutritional status in general insufficient. It leads to a significant disorder of the state of health (formed under the influence of actual nutrition) even in the presence of normal or overweight / obesity. The category of excessive nutritional status is inconvenient among other things (including the extremely widespread prevalence of overweight and obesity in the population) also because it is determined on the basis of only one excess component (accumulation of nutrient substrate) without characterizing the supply of other nutrients, which makes this category for assessment nutritional status (defined as a state of health) uninformative (it does not represent the state of the system (organism) as a whole).

An excess of certain nutrients (and excessive accumulation of adipose tissue) in a complex-systemic approach should be considered from a single position: as intoxication with these substances (or, if toxic effects are not expressed, as just pathological accumulation, for example, conditionally, "fat intoxication"), which can be superimposed on both insufficient and optimal nutritional status. The lack of nutritional deficiencies and functional impairment associated with this is defined as optimal nutritional status.

The study of the correspondence of the population's nutrition to the needs of the body, the detection and timely correction of malnutrition that increases the risk of developing health disorders is an important part of the prevention of non-communicable diseases (Bashun et al., 2020; Ragin et al., 2019; Bashun et al., 2017a), the way to reduce mortality, increase overall well-being, the duration of the painless period of life, its longevity and the preservation of human capital. The non-invasive assessment of the state of provision of particular nutrients should have the primary role as it has important advantages and is initial in this series of preventive measures and determines the strategy.

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