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Delayed Aging as a Biological Phenomenon: The Role of Biochemical and Cellular Markers

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Abstract. *This study is devoted to a comprehensive assessment of biochemical, hormonal, and cellular hematological parameters in centenarians and their immediate relatives to identify their potential role in the development of the delayed aging phenotype. The study included centenarians and their relatives living in the Goychay region of Azerbaijan, a region where longevity has not previously been studied. For comparative analysis, parameters from control groups (individuals unrelated to centenarians) were used. The study analyzed lipid peroxidation parameters and the state of the blood antioxidant system, steroid hormone levels (progesterone and dehydroepiandrosterone), and a number of cellular hematological parameters. The study results showed that centenarians develop a more favorable oxidative profile, characterized by reduced lipid peroxidation activity and relatively preserved antioxidant protection. The identified hormonal status characteristics indicate the potential role of steroid hormones in maintaining adaptive mechanisms and metabolic homeostasis during aging. Analysis of cellular and hematological parameters indicates the preservation of the functional state of the blood system, which can be considered an additional component of the healthy aging phenotype. These data confirm the importance of a comprehensive approach to studying aging biomarkers and expand our understanding of the molecular and biochemical foundations of healthy aging in humans.*

Keywords: *centenarians, aging, aging biomarkers, lipid peroxidation, antioxidant system, oxidative stress, progesterone, dehydroepiandrosterone, hematological parameters, hereditary factors, Azerbaijan*

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Gecikmiş yaşlanma bioloji fenomen kimi: Biokimyəvi və hüceyrə markerlərinin rolu

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Xülasə. *Təqdim olunmuş məqalə uzunömürlülərdə və onların yaxın qohumlarında biokimyəvi, hormonal və hematoloji parametrlərin kompleks qiymətləndirilməsinə həsr olunmuşdur. Aparılmış tədqiqatın məqsədi ləngimiş qocalma fenotipinin formalaşmasında tədqiq olunan biokimyəvi göstəricilərin mümkün rolunu müəyyən etməkdir. Tədqiqata Azərbaycan Respublikasının Göyçay rayonunda yaşayan uzunömürlülər və onların qohumları cəlb edilmişdir. Belə ki, qeyd olunan regionda uzunömürlülük səviyyəsi əvvəllər tədqiq olunmamışdır. Tədqiqatda müqayisəli analiz məqsədilə nəsildə uzunömürlü olmayan şəxslərdən ibarət nəzarət qrupunun göstəricilərindən də istifadə edilmişdir.*

Aparılmış tədqiqatlar zamanı tədqiq olunan qruplarda lipidlərin peroksid oksidləşməsi göstəriciləri və qan antioksidant sisteminin vəziyyəti, steroid hormonların (progesteron və dehidroepiandrosteron) səviyyələri, eləcə də bir sıra hematoloji parametrlər təhlil olunmuşdur. Tədqiqatın nəticələri göstərmişdir ki, uzunömürlü şəxslərdə lipidlərin peroksid oksidləşmə proseslərinin aktivliyinin azalması və antioksidant müdafiə sisteminin nisbi qorunması ilə xarakterizə olunan daha əlverişli oksidativ profil formalaşır. Hormonal statusun müəyyən olunmuş xüsusiyyətləri steroid hormonların qocalma şəraitində adaptasiya mexanizmlərinin və metabolik homeostazın saxlanılmasında potensial rolunu göstərir. Hematoloji göstəricilərin analizi zamanı isə qan sisteminin funksional vəziyyətinin qorunub saxlanılmasını aşkar olunmuşdur, bu da sağlam qocalma fenotipinin əlavə komponenti kimi qiymətləndirilə bilər. Beləliklə, tədqiqat zamanı alınmış nəticələr qocalma biomarkerlərinin öyrənilməsində kompleks yanaşmanın əhəmiyyətini təsdiqləyir və orqanizmin sağlam qocalmasının molekulyar-biokimyəvi əsasları haqqında biliklərin genişləndirilməsinə zəmin yaradır.

Açar sözlər: *uzunömürlülər, qocalma, qocalma biomarkerləri, lipidlərin peroksid oksidləşməsi, orqanizmin antioksidant sistemi, oksidativ stress, progesteron, dehidroepiandrosteron, hematoloji göstəricilər, irsi amillər, Azərbaycan*

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Introduction

A current area of research in modern gerontology is the search for and comprehensive evaluation of aging biomarkers that reflect the mechanisms of longevity and the preservation of functional reserves in old age. The classic definition of an aging biomarker was proposed by Baker and Sprott back in 1988: a biological parameter (or several parameters) that, in the absence of overt pathology, more accurately predicts functional capacity in late life than chronological age (Baker & Sprott, 1988). According to modern research, an ideal marker should reflect the fundamental mechanisms of aging, demonstrate reproducibility across different populations, be applicable across different tissues and species, respond to geroprotective interventions, and predict clinically significant outcomes (Herzog et al., 2025; Moqri et al., 2023).

One of the early attempts to systematize objective indicators of healthy aging was the work of British researchers led by J. Lara (Lara et al., 2015). The work of Lara et al. (2015) was an important step toward standardizing the assessment of healthy aging and is still cited in reviews and consensus documents on aging biomarkers. In their work, the authors proposed a panel of biomarkers focused on assessing the preservation of the body's functional capacity during aging, and not just the absence of disease (Lara et al., 2015). It should be noted that the panel of Lara et al. (2015) represents an important step in the evolution of ideas about aging biomarkers, emphasizing the priority of functional indicators over purely laboratory ones. The criteria for selecting the "ideal" biomarker have remained unchanged since the 2010s, but in 2025, a Delphi method (three rounds, 60 experts from different countries) was used to reach consensus on the biomarkers most suitable as endpoints in anti-aging studies (Perri et al., 2025). A three-stage Delphi study was conducted using an online platform. In the first round, expert panel members proposed candidate aging biomarkers. In the second and third rounds, they voted on 500 initial statements (yes/no) concerning 20 aging biomarkers. Panel members could abstain from voting on biomarkers outside their expertise. Consensus was reached when ≥70% agreement was reached on a biomarker statement. Over the course of three rounds, consensus was

reached on 14 biomarkers covering physiological (e.g., insulin-like growth factor 1, growth differentiation factor-15), inflammatory (e.g., high-sensitivity C-reactive protein, interleukin-6), functional (e.g., muscle mass, muscle strength, grip strength, Stand-up and Go test, gait speed, standing balance test, frailty index, cognitive health, blood pressure), and epigenetic (e.g., DNA methylation/epigenetic clock) domains. Expert consensus identified 14 potential biomarkers of aging that could be used as outcome measures in intervention studies. (Perri et al., 2025).

Summarizing current literature, it can be shown that the main approaches to biomarker classification can be divided into functional, molecular genetic, epigenetic, and biochemical. Currently, there are two main theories of aging: genetic and cellular damage, and neither theory can ignore the role of mitochondria. The free-radical theory of premature aging is widely accepted, positing that aging occurs due to the accumulation of cellular damage caused by free radicals over time. The accumulation of large amounts of such damage in cells leads to disruption of their normal functioning (function), followed by changes consistent with aging and leading to disease (Koltan & Medvedev, 2018). Oxygen toxicity and the activity of oxygen-reducing intermediates necessitate the constant functioning of specialized antioxidant defense mechanisms in the body, which operate at the level of molecules, cells, tissues, organs, and the body as a whole, maintaining homeostasis. The antioxidant system (AOS) is the body's most important nonspecific defense system, quickly responding to changing external conditions. The balance between lipid peroxidation (LP) and antioxidant activity (AOA) reflects the body's adaptive capacity, and its imbalance leads to pathological changes, damage to molecular and cellular structures, carcinogenesis, and death.

It is well known that one of the key aspects that attracts the attention of scientists and medical specialists is the hormonal function of the gonads and its impact on the aging process (Bets, 2013; Bulgakova & Romanchuk, 2020; Goncharov & Katzya, 1998). The body's reproductive function is one of the most fundamental and highly significant biological functions, and its decline is one of the manifestations of natural aging in humans and animals (Anisimov, 2008; Svendsen & Schultz, 2008). The activity of the reproductive system is directly related to age, and the intensity of sex hormone production largely determines a person's biological age (Bulgakova & Romanchuk, 2020; Kulikov & Arkhipova, 2021; Medzinovskaya et al., 2018).

Despite a significant number of studies devoted to individual aspects of aging, the available literature lacks data obtained on the basis of complex biochemical, hormonal, and cellular hematological parameters in centenarians and their immediate relatives (Gavrilov et al., 2020; Kim et al., 2017; Kuznika et al., 2020; Pal'tsev et al., 2009; Hammad, 2017). Similar studies conducted on populations living in various regions of Azerbaijan are particularly limited (Gashimova et al., 2019; Kadimova, 2011; Karamova et al., 2024).

Aim of the Study. Based on the above, this study is dedicated to a comprehensive assessment of biochemical, hormonal, and cellular hematological parameters in centenarians and their immediate family members. Specifically, an attempt was made to determine the informative value of certain biochemical parameters, particularly lipid peroxidation and antioxidant activity (LPO and AOA), reflecting physiological aging processes. Levels of the sex hormones progesterone and dehydroepiandrosterone (DHEA) were also studied in centenarians and their immediate family members. Serum C-reactive protein (CRP) levels, widely used in clinical and experimental gerontology as an indicator of chronic low-grade inflammation associated with aging, were also studied.

Research materials and methods. The study was conducted in the Goychay region of Azerbaijan. After determining the age of centenarians living in these regions through verification (clarification of family history, survey of historical processes specific to these regions), they were registered. All individuals classified as centenarians were registered.

The subjects were divided into three groups: Group I consisted of generally healthy men and women aged 45-50 years with no centenarians among their immediate ancestors (a total of 31 individuals); Group II consisted of elderly men and women aged 60-80 years (a total of 29 individuals); and Group III consisted of the centenarians themselves—19 individuals aged 90 years and older, including 11 women and 8 men. Characterization and comparison of the groups was based on the gender and age of the subjects.

Exclusion criteria for the study included individuals with a history of cancer, cardiovascular disease, stroke or neurological disorders, liver disease, kidney disease, gout, thyroid disease, or hormonal imbalances. Each subject completed a voluntary informed consent form to participate in the study, which explained the nature and procedure of the study, potential inconveniences and risks, and consented to the processing of personal data, taking into account the principle of confidentiality (access only to the research team and presentation of data in a general dataset). All experiments were conducted in accordance with the principles of the Declaration of Helsinki (Finland, 1964) and after review by the Ethics Committee of the Abdulla Karayev Institute of Physiology, Ministry of Science and Education of the Republic of Azerbaijan (Protocol No. 1 dated December 2, 2022).

Venous blood with the addition of heparin and EDTA anticoagulants served as the material for the study. Venous blood was collected in the morning on an empty stomach between 8:00 and 9:00 AM. Non-hemolyzed plasma, obtained no later than 2 hours after blood collection by centrifugation for 10 minutes at 3000 rpm, was used for biochemical analysis. LPO intensity was assessed by the content of primary hydroperoxides (HP) and the secondary product malondialdehyde (MDA), which was determined using the method of Asakawa T. and Matsushita S. (1980). Catalase activity in our experiments was determined using the method of Bergmeyer H.U. SOD enzymatic activity was determined using the method of Beauchamp C. and Fridovich J.

Enzyme-linked immunosorbent assay (ELISA) was used to determine serum levels of the sex hormones progesterone and dehydroepiandrosterone (DHEA) using hormonal enzyme-linked immunosorbent assay kits from CLIA/Roche Diagnostics (Switzerland). Serum C-reactive protein levels were determined using an immunoassay based on the specific binding of CRP to antibodies to it. The experimental data were analyzed using the analysis of variation statistics using the parametric Student's t-test and the nonparametric Wilcoxon (Mann-Whitney) U-test. Statistical processing was performed using Microsoft Excel (Office 2010).

Methods

When studying the GP and MDA levels in Group I individuals (individuals from families without a history of longevity), the highest levels of lipid peroxidation products were observed. Thus, the hydroperoxide content was 1.54±0.06 relative units, and the MDA concentration was 3.70±1.75 nmol/mg protein, indicating pronounced lipid peroxidation activity.

Table 1

Dynamics of levels of primary – hydroperoxides (HP) and secondary – malondialdehyde (MDA) – lipid peroxidation products in centenarians and their close relatives (M±m)

Indicators Study groups	Hydroperoxides (HP), rel. Units	Malondialdehyde (MDA), nmol/mg protein
Group I - Representatives of families where there are no cases of longevity	1,54±0,06	3,70 ± 1,75
Group II - Descendants of long-livers	1,43±0,23*	3,13 ± 0,70*
Group III – long-livers (90 years and older)	1,27±0,51*	1,58 ± 0,61*

Note: * - $p < 0.05$.

However, it should be noted that in Group II—the offspring of centenarians—relatively low levels of both primary and secondary lipid peroxidation products were observed. Thus, the GP level averaged 1.43±0.23 relative units, and the MDA concentration was up to 3.13±0.70 nmol/mg protein, which was statistically significant compared to Group I ($p < 0.05$). These changes indicate a more balanced oxidative process in the offspring of centenarians (Table 1). When studying the free radical oxidation (FRO) status in the blood of centenarians, the following results were obtained: the hydroperoxide level was 1.27±0.51 relative units, and the MDA concentration was 1.58±0.61 nmol/mg protein. These values were statistically significant when compared with those in Groups I and II ($p < 0.05$) (Table 1).

Thus, a clear age-related and genealogical trend in the intensity of lipid peroxidation processes was revealed, characterized by a decrease in the level of oxidative damage to lipids in the descendants of centenarians, and especially in individuals who reached old age. In turn, it can be assumed that the decreased MDA content in centenarians compared to the control group may serve as a prognostic marker of high vitality and act as a biochemical criterion for longevity. Moreover, high lipid peroxidation levels in the control group can be considered a risk factor for the development of age-related diseases that hinder longevity.

These data may indicate the presence of hereditary and adaptive mechanisms aimed at limiting free radical oxidation during aging. To more fully characterize the pro- and antioxidant balance, the activity of key enzymes of the antioxidant system—catalase (CAT) and superoxide dismutase (SOD)—was studied. These enzymes constitute the body's first line of defense against reactive oxygen species. The study results are presented in Table 2.

Table 2

Activity of antioxidant enzymes - catalase (CAT) and superoxide dismutase (SOD) - in centenarians and their close relatives, (M±m)

Indicators Study groups	Catalase (CAT), U/mg protein	Superoxide dismutase (SOD), conventional units/mg protein
Representatives of families where cases of longevity have not been noted	47,5 ±6,2	192,0±17,3
Group II -Close relatives of centenarians	42,1±4,7**	100,07 ± 13,4**
Group III – centenarians (90 years and older)	27,3±0,19*	95,77 ± 9,5*

Note: *- $p < 0,05$, ** - $p < 0.01$.

The results obtained in this series of studies indicate that longevity is associated not with an enhancement but with an optimization of antioxidant processes, which is reflected in a decrease in the activity of enzymatic components of the antioxidant system. This suggests that during aging, the antioxidant system functions more balanced, maintaining the body's homeostasis with lower energy expenditure.

Studies in a group of representatives of families without a history of longevity revealed the highest levels of antioxidant enzyme activity, which is likely a compensatory response to increased free-radical processes. Catalase activity was 47.5 ± 6.2 U/mg protein, and superoxide dismutase activity was 192.0 ± 17.3 conventional units/mg protein (Table 2). Blood tests in the descendants of long-lived individuals (Group II) revealed statistically significant lower antioxidant enzyme activity levels compared to the control group ($p < 0.01$). Thus, CAT activity in this group averaged 42.1±4.7 U/mg

protein, and SOD activity averaged 100.07±13.4 conventional units/mg protein. A study of the blood serum of centenarians revealed lower antioxidant enzyme activity compared to other study groups ($p < 0.05$). Thus, catalase activity in the centenarian group averaged 27.3±0.19 U/mg protein, and superoxide dismutase activity averaged 95.77±9.5 conventional units/mg protein (Table 2).

Thus, a comprehensive analysis of lipid peroxidation parameters and antioxidant enzyme activity allows us to conclude that centenarians and their offspring develop a more stable balance between the formation of reactive oxygen species and their neutralization. The combined detection of reduced activity of enzymatic components of the antioxidant system against a background of low levels of lipid peroxidation products should be considered a characteristic biochemical marker of longevity, reflecting a state of balanced and optimized free radical homeostasis. The results of the study on the characteristics of endocrine status during longevity and hereditary predisposition to it are presented in Table 3. Analysis of the obtained data revealed that progesterone levels are characterized by significant variability depending on gender and study group.

Table 3
Sex hormone levels in centenarians of the Goychay district

Indicators	Progesterone (ng/ml)		Dehydroepiandrosterone (DHEA) (µg/dL)	
Study groups	Male	Female	Male	Female
Representatives of families where cases of longevity have not been noted	0,14±0,04	0,06±0,007	65,4±7,08	17,31±3,44
Group II – Close relatives of centenarians	0,08±0,02	0,15±0,02	112,4±9,9	46,2±0,33
Group III – centenarians (90 years and older)	0,07±0,02	0,18±0,03	63,7±8,2	19,1±1,6

In men from families without a history of longevity, the average progesterone level was 0.14±0.04 ng/ml, while in women from the same group, the average was 0.06±0.007 ng/ml. Thus, in families without a history of longevity, men had higher progesterone levels than women (Table 3). When examining progesterone levels in the group of centenarian offspring, the following results were obtained: in men, this value averaged 0.08±0.02 ng/ml, while in women it was 0.15±0.02 ng/ml (Table 3). According to our data, a similar pattern of changes was observed in the centenarian group. In men, progesterone levels averaged 0.07±0.02 ng/ml, lower than control values. At the same time, progesterone levels in centenarian women reached 0.18±0.03 ng/ml, which was higher than those in all female subgroups examined. DHEA levels revealed statistically significant differences between groups and genders. Blood tests in representatives of families without a history of longevity yielded the following results: the average DHEA concentration in men was 65.4±7.08 µg/dL, while in women it was 17.31±3.44 µg/dL (Table 3).

According to our data, in the group of centenarian descendants, men showed a significant increase in DHEA levels to 112.4±9.9 µg/dL, which is almost 1.7 times higher than in the control group. In women in this group (descendants of centenarians), DHEA concentrations also increased, reaching 46.2±0.33 µg/dL, which is 2.5 times higher than the same value in women in the control group. Studies in the centenarian group revealed decreased DHEA levels in both men and women. In centenarian men, this indicator was 63.7±8.2 µg/dL, while in centenarian women it was 19.1±1.6 µg/dL, approaching values typical for members of families without a history of longevity (Table 3).

In summary, the data obtained indicate that the descendants of centenarians had high DHEA levels, regardless of gender, which can be considered a possible biochemical marker of a hereditary

predisposition to longevity. At the same time, long-lived women are characterized by elevated progesterone levels, which may indicate the role of this hormone in adaptation mechanisms and maintaining homeostasis in old age and old age. These changes in hormonal profiles confirm the involvement of endocrine mechanisms in the development of longevity and suggest this area as a promising area for further scientific research. As shown in Table 4, the average CRP level in the control group, which included members of families with no history of longevity, was 6.48±1.08 mg/L. This indicator was the highest among all the groups examined. When examining CRP levels in Group II, which included close relatives of centenarians, statistically significant low levels of this indicator were found. In this group, the range was between 3.9 and 8.5 mg/L, with an average of 5.35±0.4.

Table 4

CRP Level as an Indicator of Chronic Inflammation in Centenarians

Indicators Study groups	C-reactive protein, mg/L
Group I - control (Representatives of families where cases of longevity have not been noted)	6,48±1,08
Group II - Close relatives of long-livers	5,35±0,4
Group III – long-livers (90 years and older)	5,56±0,6

Based on our data, it can be suggested that the CRP concentration in this group may indicate the presence of certain hereditary or familial factors that contribute to the development of a more favorable anti-inflammatory profile even before reaching old age.

The results of studies conducted in a group of centenarians (individuals aged 90 years and older) showed that the average CRP value ranged from 0.5 to 8.3 mg/L, averaging 5.56 ± 0.6 mg/L and lower than control values. Thus, the study found that centenarians and their close relatives had lower CRP levels compared to members of families without a history of longevity. This indicates a reduced severity of systemic inflammation in these groups. In turn, relatively low inflammatory activity can be considered an important component of the biological mechanisms of longevity, which is consistent with current understanding of the role of inflammation in aging. The observed C-reactive protein levels in centenarians and their relatives suggest that a favorable anti-inflammatory profile is formed through a combination of hereditary and familial environmental influences and is associated with longevity.

Results and discussion

The results of this study indicate that delayed aging and longevity are due to the development of a specific set of biological characteristics reflecting the optimal functioning of the body's regulatory systems. Centenarians and their immediate relatives exhibited a more favorable balance of pro- and antioxidant processes, characterized by reduced lipid peroxidation and balanced activity of antioxidant enzymes. These changes indicate not hyperactivation of defense mechanisms, but their efficient and functionally effective regulation.

Hormonal status characteristics, in particular variability in progesterone and dehydroepiandrosterone levels, confirm the involvement of endocrine mechanisms in maintaining the body's adaptive capacity and metabolic homeostasis during aging. Furthermore, reduced chronic inflammation, reflected by lower C-reactive protein concentrations, can be considered a key factor associated with longevity.

Taken together, the obtained data allow us to conclude that longevity is formed through the interaction of hereditary and adaptive regulatory mechanisms that ensure the stability of the body's internal environment in the face of age-related changes. A comprehensive analysis of biochemical, hormonal, and inflammatory parameters confirms the feasibility of their use as informative markers of delayed aging and identifies prospects for further research in the field of the molecular and physiological foundations of healthy human aging.

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