The Scientific Basis for Healthy Aging and Antiaging Processes

New York, 2011

Editors

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List of abbreviations and definitions

ACE Angiotensin converting enzyme

ACS Acute coronary syndrome

ACTH Adrenocorticotropic hormone

AD Alzheimer's disease

AGT Angiotensinogen

AGTR1 Angiotensin II receptor, type 1

AGE Advanced glycation endproducts

ALOX Arachidonate lipoxygenase

AMPA Alpha-amino-3-hydroxyl-5-methyl-4-isoxazole-propionic acid

AMPA-receptors Ionotropic glutamate receptors regulating the permeability of ion channels, sensitive to AMPA (alpha-amino-3-hydroxyl-5-methyl-4-isoxazole-propionic acid) action

AMPK 5'AMP (adenosine monophosphate)-activated protein kinase, cellular protein kinase controlling energy balance of a cell

APOA1 Apolipoprotein A-1

APOB Apolipoprotein B

APOE Apolipoprotein E

ATCC American Type Culture Collection

ARG Regulatory aging gene

BAFS Biologically active food supplements

BWI Body-weight index

CAT Catalase

CETP Cholesteryl ester transfer protein

CD Clusters of differentiation

CR Caloric restriction

CR Cardiac rate

CREB cAMP response element-binding protein

CRP C-reactive protein

CS Cholestyrine

CYP2D6 Cytochrome P450 2D6

CYP17 Cytochrome P450, family 17

CYP19 Cytochrome P450, family 19

C12TPP Analogue of SkQ lacking plastoquinone

CVD Cardiovascular disease

DBP Diastolic blood pressure

2DG 2-Deoxyglucose

DVS Direct inoculation technology

DHEA Dehydroepiandrosterone

DOPA Dihydroxyphenylalanine, physiologically active compound, producing intermediate product in the process of catecholamine synthesis (dopamine, adrenaline and noradrenaline)

ECG Electrocardiogram

EH Essential hypertension

EHEC Enterohemorrhagic *Escherichia coli* (*E. coli*)]

ESC Embryonic stem cells

HER2 Oncogene

HLA Human leukocyte antigen

HSP Heat-shock protein

HGF Hepatocyte growth factor

FAD Flavin adenine dinucleotide

FOXO Forkhead box class O

FSH Follicle-stimulating hormone

GABA Gamma-aminobutyric acid

GH Growth hormone

GHR/BP Growth hormone receptor/binding protein

GIT Gastrointestinal tract

GnRH Gonadotropin-releasing hormone

GSTM1 Glutathione S-transferase Mu 1

GSTT1 Glutathione S-transferase Theta-1

HDL High-density lipoprotein

HHD Hypertensive heart disease

IBD Inflammatory bowel disease

IBS Irritable bowel syndrome

ICA Individual chronological age

IFNγ Interferon gamma

IGF Insulinlike growth factor

IGF-1 Insulin-like growth factor-1

IHD Ischemic heart disease

IL-1β Interleukin-1β

IL-2 Interleukin-2

IL-7 Interleukin-7

IL-6 Interleukin-6

IL-2 Interleukin-2

IL-10 Interleukin-10

iPS-cells Induced pluripotent stem cells

IRS Insulin receptor substrates

KGF Keratinocyte growth factor

LAB Lactic acid bacteria

LDL Low-density lipoprotein

LE Life expectancy

LP Lipoprotein

LPA Lipoprotein A

MAO Monoamine oxidase

MCA Middle chronological age of examined persons

MI Myocardial infarction

MTHFR Methylenetetrahydrofolate reductase

MTP Microsomal triglyceride transfer protein

mTOR Mammalian target of rapamycin

MitQ Mitochondrial antioxidant (10-(6′-ubiquinonyl) decyltriphenylphosphonium)

mtDNA mitochondrial DNA

NADH Nicotinamide adenine dinucleotide

NAT2 N-acetyltransferase 2

NGF Nerve growth factor

NIDDM Non-insulin-dependent diabetes mellitus

NK Natural killer (referring to a distinct population of lymphocytes)

NMDA-receptor (NMDAR; NMDA-receptor) ionotropic glutamate receptor selectively binding N-methyl-D-aspartate

PAI1 Plasminogen activator inhibitor type 1

PGC-1 Coactivator PPAR-γ 1 (peroxisome proliferator-activated receptor γ1)

PI3K Phosphoinositide 3-kinase

PIT1 Pituitary-specific transcription factor

PDGF Platelet-derived growth factor

PON Paraoxonase

PPARα Peroxisome proliferator-activated receptor alpha

PPARγ Peroxisome proliferator activated receptor γ

PSA Prostate-specific antigen

ppm parts per million (units of concentration)

RBA CVS Reference biological age of the cardiovascular system

REN Renin

ROS Reactive oxygen species

rRNA Ribosomal ribonucleic acid

SBP Systolic blood pressure

SCFA Short-chain fatty acids

SIRT1-7 Sirtuins: a class of proteins having properties of histone deacetylase and monoribosyltransferase

SDR Short-chain dehydrogenase/reductase

SIRT-1 Silent information regulator-1

SOD Superoxide dismutase

SOD2 Superoxide dismutase 2

SkQ Mitochondrial antioxidant 10-(6′-plastoquinonyl) decyltriphenylphosphonium

STACs Sirtuin activators among pharmacological drugs

STEC Shiga toxin-producing *Escherichia coli*

TG Triglyceride

TGF Transforming growth factor

TGFβ Transforming growth factor-beta

TK Tyrosine kinase

TLR-4 Toll-like receptor-4

TNFα Tumor necrosis factor-alpha

TOR Target of rapamycin

TPA Tissue-type plasminogen activator

tPA Tissue plasminogen activator

UGT UDP- uridine diphosphate glucuronosyl transferase

β-Site APP-cleaving enzyme (BACE) An enzyme belonging to the group of membrane-bound aspartyl proteases and involved in the production of Aβ amyloid peptides in Alzheimer's disease

RES Reactive oxygen species

Foreword

Aubrey D.N.J. de Grey, Ph.D.
Chief Science Officer, SENS Foundation
Editor-in-Chief, *Rejuvenation Research*

Is aging a disease? Experts and commentators alike have produced vast amounts of debate on this question, but ultimately it is no more than a matter of semantics. Aging is bad for you, and results in debilitation and death, so it is indisputably a potential target for medical intervention. For far too long, however, this has been an unpopular thing for those who study aging to mention—at least, to mention publicly. But that is changing—and not only in the Western world.

In this volume, a group of researchers from a nation that has been hitherto discreet in this field has put together a highly impressive survey of both the current state of knowledge about aging and the prospects for postponing it with present and future medical interventions. In the following pages, they survey the past and future demographic consequences of increasing life expectancies and the social and environmental impact thereof; the various mechanistic hypotheses for what drives the aging process; the measurable changes with age that best serve as indicators of remaining healthy longevity; the simple (mainly small-molecule) interventions currently available that may delay the ill-health of old age; the gerontological relevance of the interaction between the body's own cells and the far more numerous cells populating our gut; and the current and future prospects for the comprehensive medical postponement of age-related ill-health. This book presents a comprehensive overview of all major topics relating to the elevation of aging to its rightful status as a phenomenon to be tackled by medicine.

Why is a book of this nature needed? I would like to highlight two main reasons, though there are certainly others. First, it updates previous surveys of the field that have been penned both by individual authors (Comfort, Finch and Arking come to mind) and by consortia organized by influential editors such as Masoro and Austad; frequent such updates are undoubtedly needed, and increasingly so, as the field broadens and accelerates. But perhaps an even more important reason for this book is its geographical origin. Aging is too easily viewed as a problem restricted to the industrialised world, in which the most noticeable causes of disease and death in other regions are things of the sufficiently distant past that most of us have essentially forgotten that those causes ever afflicted us. But the developing world is called "developing" for a reason: it is following the developed world into prosperity. And that prosperity is rapidly translating into a shift of such nations' health problems, into exactly the ones that have dominated the developed world since WWII—namely, the problems of aging. The countries whose prosperity is rising most rapidly are also those whose "aging problem" is increasing the fastest. The most populous such nations—China, India and Brazil are all seeing a plummeting fertility rate and mortality rate at older ages, and also the inevitable consequence of a rapidly increasing average age and a huge rise in the proportion of the population suffering from age-related disease and disability.

What of smaller nations? Kazakhstan does not make many headlines as a leading nation among those transitioning to "developed" status, but it soon may. Its intrinsic wealth is an important factor, but so is its endeavour: Nazarbayev University, named after Kazakhstan's head of state, is devoting considerable energy to research in the biology of aging. Also, though smaller than the aforementioned countries, Kazakhstan is far from small: its resources,

in terms of both finances and personnel, are ample to make a real difference to the global movement against aging. Accordingly, I am delighted that this small but rapidly growing group of researchers is not only pursuing valuable research, but is also making its presence felt within the international research community, not least via this book. I sincerely hope and expect this text will serve both as a valuable resource to biogerontologists worldwide, and also as a powerful advertisement that Kazakhstan has well and truly arrived on the biomedical gerontology scene.

Preface

Alan J. Russell, PhD
Distinguished University Professor & Founding Director
McGowan Institute for Regenerative Medicine
University of Pittsburgh & UPMC

The question of how to live longer, more fulfilling lives has driven the lights and shadows of science for generations. Leaders of powerful countries and regions have had to make difficult decisions about how to achieve certain rights we all desire: to be free and healthy. Regrettably, we cannot live forever, and reaching the equilibrium of a long, healthy life and the decay of old age often proves difficult. In Kazakhstan, the exploration of what leads to longevity and aging is being addressed by diligent researchers, great minds, and powerful science and technology. In this book, we delve into the exciting new science and explore the great strides being made in search of finding a balance between longevity and the natural course of life and death.

By using its wealth and natural resources, Kazakhstan has successfully been able to improve the lives of its citizens. Many countries share in this dream, but far fewer have the capacity to change their course in the space of a generation. Kazakhstan has developed an integrated strategy to use education, science and engineering to deliver longer and better lives to its people. With the extraordinary vision and commitment of President Nazarbayev, combined with resources from the oil and gas industry that have the potential to be a dominant force, scientific leaders in the region have launched a Center for Life Sciences that stands at the center of Nazarbayev University. Astana will follow the path of the major Western cities, like Manchester, Munich, and Madrid, that have found a way to shed the yoke of the past and develop a vibrant free technology based culture. The focus of the Center for Life Sciences has been the science of regenerative medicine and healthy aging. Under the expert leadership of Almaz Sharman, MD, PhD, and Zhaxybay Zhumadilov, MD, PhD, this volume of work emerges from the vision and determination of powerful minds and innovative research being explored at the Center for Life Sciences, and is the starting point of a journey that is being propelled by the President of Kazakhstan.

Delivering healthy aging to patients requires a detailed understanding of how cells and tissues degrade over time and how to favorably intervene in that process. This book presents a detailed faculty-level perspective of an emerging center of excellence and the biology of aging at the cellular level. Regenerative medicine through cell therapy, artificial organs or tissue engineering, all alter today's paradigm of how we treat disease. Today we generally develop therapies and surgical strategies that ameliorate symptoms—tomorrow, through the kind of science described in this book, we will be able to offer cures by restoring tissue and organs that have lost form and function through injury and disease.

In the next ten years, the growth and achievement of the Center for Life Sciences will undoubtedly produce another volume reporting incredible advancements in the field. Increasing longevity in Kazakhstan can be measured in terms of success far more easily than the success of a handful of science projects. The commitment of the authors of this book is to the citizens of their country as much as it is to the impressive nature of the science. Their passion for the future will be rewarded.

Chapter I

Demographic Transformations, Perspectives on Longevity, and Challenges for Health

ALMAZ SHARMAN, MD, PHD, D.M.SC.,

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Nazarbayev University

Both the Roman philosophers Cicero and Publius Cornelius and the medieval Arab philosopher IbnKhaldun believed that increased life expectancy is a feature of civilized countries and evidence of a high quality of life [1–3]. In less than two decades, Kazakhstan has managed to create a powerful, independent, and internationally recognized state with a harmonious and stable society, and is now working on the long-term goals of improving the quality of life and increasing longevity of its citizens [4].

The matter of prolonging life should be considered both individually and in a social context. For the individual, longevity is not simply a large number of years lived, but rather years of active and high-quality life free of exhausting chronic diseases, memory loss, and other ailments. Longevity and quality of life are strategic investments in human capital, which in the modern world are considered the most forward-looking and long-term preconditions for an innovative and competitive economy. However, it is important to recognize that both individual and social aspects of increased longevity are associated with unprecedented challenges for health and social services that have already been challenged by many technological, scientific, ethical, and other problems. All of them are related to the demographic transformation.

1.1 DEMOGRAPHIC CHANGES IN THE TWENTY-FIRST CENTURY

It wasn't a long time ago that demographers warned us about the threat of unprecedented population growth. Which, they argued will get out of control and lead to food shortages and widespread hunger.

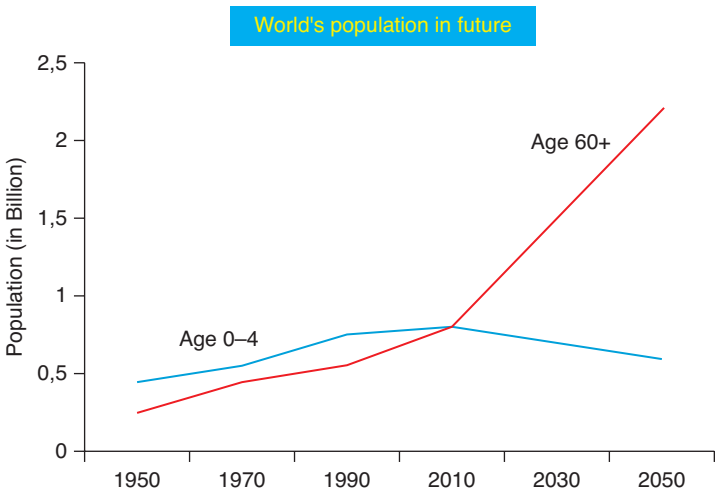

Figure 1–1.

But in reality, the Malthusian theory of population growth as a geometric progression turned out to be wrong [5]. Although the world population continues to grow, its growth is different from what was predicted by demographers of the past century. According to a United Nations forecast, the world population will increase by 40% in the next 40 years: from 6.9 to 9.1 billion [6]. But the nature of this increase will be fundamentally different from what humankind has experienced in the past. The world's population will not grow because of high birthrate as it always did previously, but mainly as a result of increased numbers of older people. By the mid-21st century, the world population of children under the age of 5 years will decrease by 49 million while the number of people over the age of 60 will increase by 1.2 billion [7].

One of the reasons for such redistribution is the phenomenon called "baby boom." This stems from the original baby boom in the late 1940s and early 1950s, when the veterans of the Second World War returned home. An echo of this phenomenon has since recurred every 25 to 30 years as children born in those postwar years (baby boomers) produce their own offspring. For example, there was a baby boom in the mid-1970s. The present generation is witnessing a new wave of increasing birthrates. The next wave is expected soon after 2020 [8].

However, in the past just the share of the babies in the population increased but very soon we will see a similar increase in the number of people 60 years of age and older. In the long-term future, a significant increase in the number of those over 80 years of age is predicted. Baby boomers who want to live a long quality life are begining to overcrowd the world [9]. The problem with this is that this generation is relatively unconcerned about the importance of reproduction on stustaining the population level. In order for the population of any country to continue, the average family must have at least two children. But in Japan, an average woman has only 1.25 children. An interview of young women in Austria has shown that the average number of children wanted per family is 1.5, which is sufficient for "reproduction" of the mother herself but not of a spouse [10, 11].

This tendency of birth rates to fall below the "replacement rate" began in 1970 in the Scandinavian countries. Today it has spread to 59 countries in Europe, Asia, South America, and the Middle East. In the forefront of this trend are the southeastern Asian nations of South Korea, Taiwan, and Singapore, where a decrease in population is expected over the next 15 years. Interestingly, 18 of the 59 countries with an insufficient population replacement rate are categorized as developing nations [12]. The population of the Russian Federation is today 7 million less than it was in 1991.

In the past, the population of most developing countries increased largely because of a high birth rate, which only partially was offset by the high infant mortality rate. However,

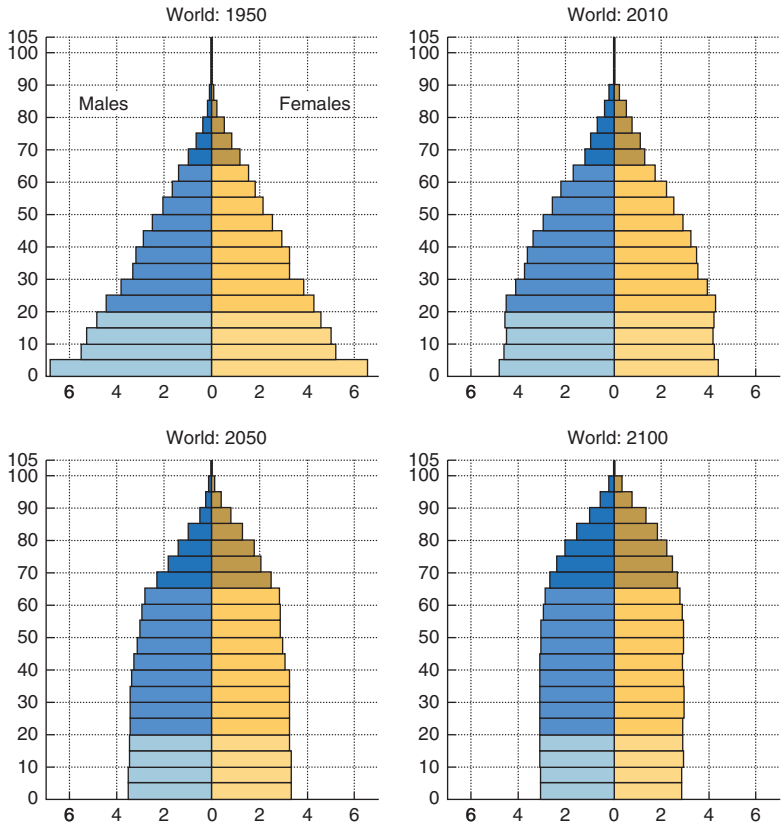

Figure 1–2. Demographic transformation models [7].

in the 1970s, developing countries saw decreased infant mortality. Simultaneously, many of these countries have witnessed a significant decline in birth rate. Consider Iran, for example. In the late 1970s an average Persian woman gave birth to 7 children. Yet today, the average woman in Iran has on average only 1.74 children. Not only is such a trend occurring in Iran; women in many developing countries now have fewer children than necessary for sustaining population.

Throughout human history, population resembled a pyramid, in which young children were the majority and formed the base of the pyramid, and the subsequent population brackets getting smaller, with the topmost and smallest component of the pyramid consisting of 70- to 80-year old people. In the 1950s, for example, children under 5 years of age represented more than 10% of the populations of developed countries; adults aged 45 to 49 represented 6%, and adults over 80 years of age represented only 1%. Today in many countries the number of 5-year-olds and 45-year-olds is about the same. In the near future, the number of people over 80 years of age will be about the same as the population of children under 5 years of age. In other words, what is now a population pyramid will be gradually transformed into a rectangle (Figure 1–2).

One can predict that over the next decade, the world's population growth will slow significantly, and after that the population will be significantly reduced. A recent study published in *Nature*, one of the most prestigious scientific journals, anticipated a significant reduction in the world's population by 2070, with an estimated world population in 2150 of half its current size. Much of this population is expected to consist of people older than 60 years of age [13].

1.2 INCREASES IN LIFE EXPECTANCY

For almost the entire history of human development, save for the past 100 years, the average individual life expectancy was not more than 30 years. This was due mainly to high infant mortality, with every fourth child dying of infectious diseases and other causes before the age of 5 years [14].

Attention to many causes of infant mortality led to a significant increase in life expectancy in the developed world. By the 1970s less than 2% of children in developed countries died before the age of 5, and as a consequence the average life expectancy has increased to an average of 70 years. Its further increase now depends on the prevention of heart and pulmonary diseases, stroke, diabetes, and cancer among people of middle and older age. With this approach, it can be expected that the average 65-year-old man will be able to live an average of twenty more years and considerably longer in the future [7, 15].

The average human life expectancy in the world has been increasing steadily, rising by an average of 6 hours per day. The life expectancy of people aged 65 years continues to increase significantly, and the prospects of increasing longevity for those who reach their 85th birthday are even better. Thus, the longer a person lives, the greater the likelihood that he or she will live even longer. The truth is simple: people who prone to chronic diseases and whose lifestyles are associated with an increased risk of disease and injury fall out of the cohort of long-lived individuals at an early point. Their health and survival are poorer than that of the remaining population.

The population of Kazakhstan is projected to grow to 21 million by 2050 (Table 1–1). The dynamic of the country's aging population resembles trends elsewhere in the world (Figure 1–3), with a projected slight but steady growth of population groups over the ages of 65 and 80 years.

Kazakhstan's President, Nursultan Nazarbayev, has set a goal of reaching an average life expectancy in Kazakhstan of 70 years by the 2015. To reach this goal it will be important to address two fundamental issues. First is the increase in life expectancy that will occur through a further reduction in infant and child mortality, and second is the promotion of longevity through adherence to a healthy lifestyle, disease prevention, the introduction of modern diagnostic and therapeutic technologies for the chronic, noncommunicable diseases prevalent in Kazakhstan.

The first problem has already largely been solved through programs promoted by the World Health Organization and the United Nations International Children's Education Fund (UNICEF), such as safe motherhood and the integrated management of childhood illnesses. These programs have proven successful in many countries around the world, and there is a strong potential for increasing life expectancy in Kazakhstan by reducing infant and child mortality through the further adoption of successful international programs and technologies.

The development and adaptation of technologies aimed at reducing infant mortality may contribute to the goal of an increased life expectancy. The National Medical Holding, a clinical

Table 1–1. Population growth in Kazakhstan by age group (in thousands)

	1950	*1970*	*1990*	*2000*	*2010*	*2020*	*2030*	*2040*	*2050*
Total	6703	13110	16530	14957	16026	17680	18873	20048	21210
<15	2303	4925	5202	4135	3925	4867	4471	4465	4866
15–64	3962	7479	10361	9802	11015	11410	12361	13156	13455
65+	438	706	967	1020	1087	1403	2041	2427	2889
80+	52	114	185	153	193	263	270	487	625

World Population Prospects, 2010 Revision

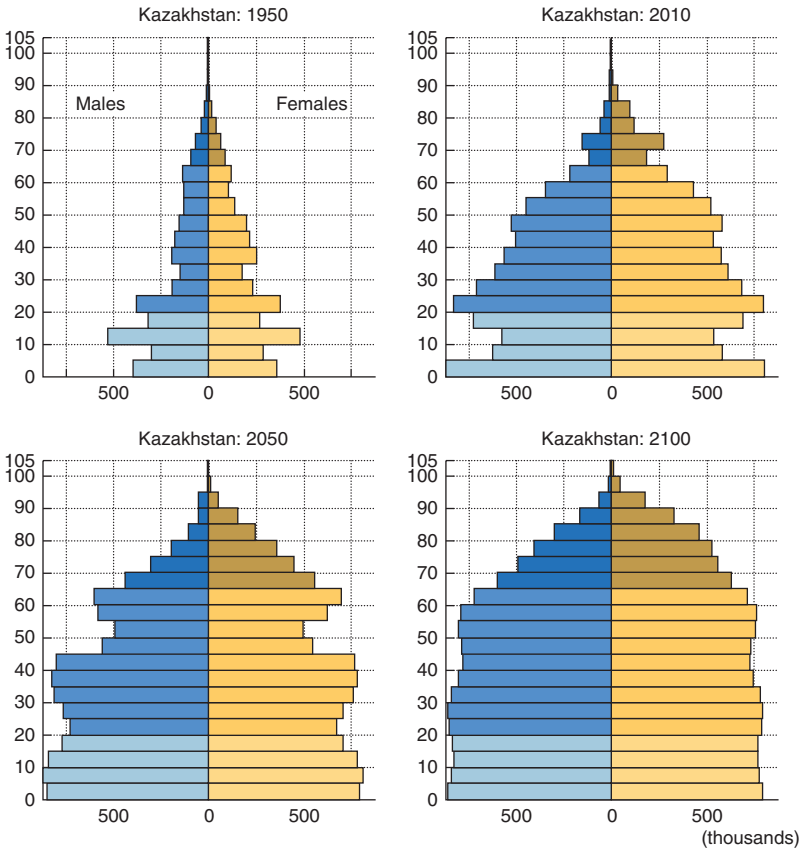

Figure 1–3. Demographic trends in the Republic of Kazakhstan.

research center at Nazarbayev University, conducts experimental and clinical research on fetal surgery. Addressing this technology, the President, in his lectures to students at the University pointed out that the introduction of surgery that allows to cure illness and disease in the fetal period is important for reducing infant mortality and increasing fertility rates in the country, and is desperately need [16].

With regard to the prevention and treatment of diseases of adults, especially among older young adults, the middle-aged, and the elderly, the situation is more complicated. Aging is associated with a significantly increased risk of chronic autoimmune diseases and cancer. Reducing this risk requires solving numerous technological, infrastructural, social, and resource issues. The physician's task is not only to treat disease but to maintain the quality of patients, lives, which is defined as achieving the maximum possible freedom from disease and the preservation of functions sufficient for active participation in everyday life.

1.3 LONGEVITY AND CHALLENGES FOR HEALTH

Today the provision of health care in many countries is facing unprecedented challenges. They are related to demographic changes, the globalization of the world economy, the emergence of new and re-emergence of old infectious and noncommunicable diseases, and environmental issues, as well as the necessity for restructuring the medical-education system and

infrastructure for the delivery of medical care. Compounding these challenges are high costs of medical technologies and the frequent lack of an evidence base for the selection of specific diagnostic and treatment methods for various diseases. High demand for expensive specialized care, especially in the elderly population, as well as an excessive focus on treatment rather than on prevention and proactive medicine are among factors contributing to the skyrocketing costs of health care [17].

In many countries, these challenges are still further complicated by the fragmentation of systems of health care, which adversely affects the continuity of care [18].

The process of developing and introducing new methods of disease diagnosis and treatment is often lengthy, costly, and ineffective. Scientific discoveries are rarely translated into real clinical practice.

Another challenge is related to technological difficulties in designing new drugs and therapies. Today, the requirements for new drugs are much more advanced and complicated than in the past, when it was sufficient to develop a new chemical substance or formula that was effective in easing an illness. Modern pharmaceutical production is a complex biotechnological process that uses molecular-genetic and other technologies that demand a rigorous scientific approach in design, use, and safety, as well as extensive knowledge and experience in drug design and application. The low hanging therapeutic fruits of medical science have already been picked off. Reaching for the remaining fruits, located at the top of the tree of knowledge, requires both greater effort and greater resources.

It gets worse. Not only it is difficult to create new drugs and medical technologies, but to apply them in clinical practice requires vast knowledge. Currently, about 6000 different kinds of medicines and 4000 surgical and other interventions exist for more than 14,000 known diseases. The physician's task is to use this armory and other resources in an accurate and timely manner in each case of illness in each patient. This requires deep and comprehensive knowledge and experience [19, 20]. There is no other industry in the world in which more than 14,000 different production lines are used. Yet such is the position of modern medicine, which, thanks to science, is constantly being improved and developed. And to remain at the forefront of modern medicine, it is vital to correctly use scientific knowledge, for which it is best to be directly involved in scientific research.

This last demand is why one of the priorities of Nazarbayev University is the integration of medical research, education, and clinical practice. It is achieved through an Integrated University Academic Healthcare System that comprises clinics of the University's National Medical Holding and Center for Life Sciences, and which in the future will include its School of Medicine. The synergistic interaction of these structures of Nazarbayev University is directed at making an important contribution toward solving a range of major challenges to health care, especially in the context of the demographic transformation described above.

Nevertheless, it would be inappropriate to rely only on technological innovation and the capabilities of medicine for the improvement of health and longevity. In discussing human health and longevity, the metaphor of the need for periodic maintenance and updating of a house seems most appropriate. Even if it is solidly built, it is unknown how long a house can withstand the numerous climatic effects of rain, winds, heat, and cold. If nothing is done, the roof will begin leaking, rain water will penetrate into the house, and the house will gradually begin to deteriorate from water and wind. Yet if the damage is promptly removed and the damaged parts replaced with new and better materials, the house can serve many future generations.

This applies to our bodies too. The only difference is that although we more or less clearly know the materials that should be used to repair a house, our knowledge of the biological principles of life is still very limited. Fortunately, the past decade has been characterized by major breakthroughs in understanding the internal structure of the body, with much greater and progressively increasing knowledge about our genes, cells, and tissues; musculoskeletal,

respiratory, and cardiovascular function; and the function of the brain and nervous system, as well as about what happens when the body malfunctions [21].

With this, we have begun to realize that disease and aging are not just relentless and unavoidable phenomena. It is quite possible to manage and control our health through adherence to a healthy lifestyle timely detection of malfunction in the body, and a use of modern medical techniques to correct abnormal functions. All of this depends basically upon ourselves.

The next chapter presents the basic theories of aging and describes the processes that occur in the human body during aging. Longevity does not mean only long life, but rather an active life even at old age free from diseases and illness. This is a recent view and a radical one in terms of traditional and historical concepts about life. The idea that old age is a plastic concept, rather than natural and immutable one, is itself a completely new paradigm.

Today scientists argue about the main causes of aging and how to deal with them. So far, science has not given us any clear answers to this question. But do we need to wait until we have a definitive answer? Even today we can take right steps toward a long and active life, free of diseases and maladies. Much of the answer to how long we will live is in our actions rather than our genes.

1.4 HEALTHY BEHAVIOR AND THE HORIZONS OF LONGEVITY

Currently, health is most often understood as the result of disease control. We teach doctors how to help patients, we build and equip hospitals, and we work to cure disease. Yet we are still forced to defend ourselves against the onslaught of many diseases. Clearly it would be better not to continue such a defensive posture but to instead take an offensive position against disease by preventing it whenever possible and detecting and treating it at an early stage when it does occur. Illness is often the result of our ignorance and unwillingness to prevent it.

The prevention of disease and prolongation of healthy years is not a very difficult task if one follows fairly simple rules, such as engaging in regular physical exercise, consuming a sufficient amount of clean water, maintaining balanced nutrition, keeping ones internal and external environment clean, early diagnosing and prevention of diseases, as well as having a positive emotional attitude and socializing with friends and relatives [22–26].

A recent study published in the *Journal of the American Medical Association* demonstrated that even at older ages, changes in lifestyle that include starting regular physical activity and proper nutrition can prolong life. The scientists who conducted the study followed 15,700 people, aged 45 to 64 years, over a 10-year period. They noted that 970 of these people decided to change their lifestyle in the sixth year of the study, beginning to exercise for an average of 3 hours per week, eat fruits and vegetables five or more times a day, stop smoking, and lose weight. After four years of such change it became clear that mortality among the study subjects who did this was 40% lower and their incidence of heart disease 35% lower than those of the subjects who continued with an unhealthy lifestyle.

On the basis of fairly simple principles of disease prevention, some developed countries have adopted national strategies and specific target indicators for extending the lives of their citizens. In the United States, 635 such target indicators were developed under the government initiative called "Healthy People 2010." For example, it set such goals as increasing the number of people engaged in physical activity to 80%, reducing smoking by at least 12%, and keeping a ceiling of 40% for the population with excess body weight. Priorities for achieving these goals included increasing the percentages of people regularly consuming fruits to 70% and those consuming vegetables to 50%, and increasing the percentage of people consuming less than 2.5 grams of salt to 65%. An important remaining goal is decrease the prevalence of diabetes to 25 persons per 1000 population. Although many of the goals of the Healthy People 2010 initiative have

still not been met, its results show a clear motivation on the part of the government and most citizens of the United States to live a long life of high quality [27].

1.5 LOW FERTILITY AND AN AGING PLANET: GOOD OR BAD?

The relationship between the material well-being of society and demography is cyclical. Initially, a decline in birth rate could mean the allocation of more resources to the individual upbringing and education of a child. This may stimulate the economy. In the 1960s and 1970s, Japan experienced such a trend, and as noted earlier, several countries in sotheastern Asia, as well as China, are experiencing this trend today [28].

Yet with the passage of time, the benefits of such a trend are not so promising. The fact is that a low birth rate does not simply mean fewer children. In the long term it means a reduction in the number of potential consumers in an economy. Fewer young people also means fewer home buyers and fewer purchasers of furniture and other goods, as well as fewer people who are willing to risk starting new businesses.

Given these circumstances, some countries have taken serious measures to boost their birth rates. A decade ago, the government of Sweden initiated an ambitious program providing financial incentives to women who willing to have more children. The government of Singapore offers women 3,000 Singapore dollars for their first child and 4,500 dollars for subsequent children, in addition to providing paid maternity leave and other material incentives for bearing children. Similar measures are now taken by the Government of the Russian Federation. So far, however, the results of such measures are not sufficiently effective and promising.

The problem hampering such efforts is probably that most births are regarded not only as a family joy but also as a serious economic liability and in some cases as a burden to a family. The days when a newborn was considered an investment and an addition to a family's labor resources are gone.

Currently in Kazakhstan, the birth rate is at a normal level—2.5 children per woman of reproductive age. However, as see in Table 1–2, the current trend indicates that the birth rate will decline in coming decades.

Statistics also demonstrate a clear trend toward an increase in average life expectancy in Kazakhstan. By the year 2050 the life expectancy is likely to reach 74 years, based on a longevity of 78 years for women and little more than 70 years for men. It therefore seems that to some extent, Kazakhstan is following a trend similar to that in many developed countries of a declining birth rate and increasing life expectancy, with all of the societal and economic implications of this.

An alternative solution to demographic problems is the effort to improve the quality of life and the ability of the older population to maintain employment. Today, the prolongation of life should not simply be an addition to the lifespan of years of senility marred by debilitating

Table 1–2. Demographic trends in Kazakhstan

	1950	*1970*	*1990*	*2000*	*2010*	*2020*	*2030*	*2040*	*2050*
Birthrate	4,41	3,67	3,03	2	2,54	2,11	1,95	1,85	1,85
Average life expectancy*	55	61,5	67,4	63	65,8	68,9	71,2	73,1	74,6
Males*	50,2	56,3	62,4	57,5	60,2	63,8	66,5	68,8	70,6
Females*	60,6	66,7	72,1	68,9	71,5	73,9	75,6	77,1	78,4

**Agency for Statistics, Republic of Kazakhstan [29, 30]*

chronic diseases, memory loss, and other ailments. It should consist of an extension of years active, healthy, and high-quality life.

A recent European Commission study showed that the creation of part-time jobs contributed to a significant extension in retirement age as well as to an increase in fertility. This is probably the result of decreased tension in the choice of work versus family life as a priority. Further important effects of such part-time work were the creation of conditions promoting physical activity and healthy eating [31].

Today's generations are aging differently than previous generations. The physical and mental health of today's 65-year-olds resemble those of 50-year-olds living in the middle of twentieth century. In the past, fewer people thought about their health—smoking, alcohol consumption, and poor nutrition were common. The country needed economic growth, which was achieved at the cost of ignoring its natural ecosystem, which was polluted with toxic industrial waste. The result was that today, the population of Kazakhstan must deal with many chronic diseases, including cancer, diabetes, and heart and lung disease. Nevertheless, the older population is less affected by disability than previous generations. Whether this trend continues will depend primarily on the extent to which the older generation engages in a healthy lifestyle.

President Nursultan Nazarbayev of Kazakhstan has set a very realistic and fundamental goal for increasing the life expectancy of the country's population, of increasing the number of quality years in life of its people. This can be accomplished through public investment in innovative research technologies directed at developing new methods of disease diagnosis, treatment, and prevention. Much will depend on scientific discoveries of the causes of chronic diseases and aging, and on the development of effective measures for counteracting them.

Societies are interested in the quality life of their citizens. But no government doctrine or investment can improve human health unless people themselves are genuinely interested in this. Healthy aging is the responsibility of individuals, families, and the wider society of which they are a part.

Immortality is a centuries-old dream of humanity reflected in many epics poems and legends. The Sumerian King Gilgamesh became famous for making an epic journey in search of eternal life. He concluded that although immortality remained for him an unattainable dream, a man should do everything possible to extend the years of life allotted to him.

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Chapter II

Theories of Aging and Its Biology and Physiology

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The analysis of numerous theories of aging reveals considerable change in the viewpoints of research scientists as a consequence of scientific progress and practical achievement. A trend from purely theoretical and hypothetical discourse toward scientifically proven approaches to aging is clearly evident. These latter approaches have allowed many investigators to propose specific measures for extending life and preventing diseases associated with aging. Of particular note is that the major achievement of research in this area is the conceptual understanding that the aging of living organisms is not a pre-programmed process of senescence but a complex process of adaptation and regulatory changes in response to environmental change and stress. Controlling these regulatory changes can prevent pathologic conditions that aggravate the aging process, thereby improving the quality of health in later life. While early theories of aging were based on organ or system-level damage to an organism, subsequent theories have been based on cellular and molecular-genetic mechanisms. This in turn has had the effect of reducing differences in theories of aging in favor of complementarities of such theories with one another, especially since so far there isn't a fully integrative concept of post-reproductive ontogenesis that would explain all of the systemic processes responsible for all aspects of aging and facilitate the development of integrated programs for improving the quality of life and anti-aging. The development of an integrated concept of postreproductive ontogenesis would have great scientific and practical value, especially in view of recent achievements and new knowledge in the areas of genomics, proteomics, and regenerative medicine. The concept of postreproductive ontogenesis, based on the synergistic and antagonistic interactions of various processes already known to genetically and phylogenetically promote reproduction and development, assumes a reorganization of evolutionarily developed regulatory processes favoring adaptation of the organism to adverse internal and external effects after decrease in reproduction function. Thus, the strengthening and reprogramming of some functions

can compensate for the loss of others, and natural disruption in postreproductive development can strengthen or weaken processes causing various types of aging. The study of these functions and the factors that affect them both favorably and unfavorably will lead to the understanding of many issues in human ontogenesis and aging.

Before considering the basic theories of human aging, however, it is important to define the key aspects in any possible theory of aging. These are defined by answers to these questions: What is aging? Why does the human body get old? Clearly, there are no unambiguous answers to these questions. However, they can at least initially be addressed through hypotheses. The organization named "Science for the Prolongation of Life" recently sponsored a discussion of basic issues in the biology of aging, in which some leading gerontologists participated. Key questions raise, and the answers received [1] are given below. They appear to reflect the most popular viewpoints about the processes of human aging and of the factors that must be addressed for modifying this process.

What is aging?

1. A process marked by an increased probability of death for biological reasons.
2. The execution of a biological program of self-destruction.
3. Disruption of homeostatic balance at different levels of organization of a living system as the result of an age-dependent decrease in the functionality of internal support systems.

Why do organisms experience a progressive and irreversible reduction in physiological function during the last period of their lives?

1. They have an endogenous program for aging.
2. They experience a catastrophic accumulation of random damage.
3. They experience damaging effects of pleiotropy, which constitute a quasi-program for aging.

We will consider the most popular theories of aging with consideration of these three key points. To date, more than 300 theories have been offered to explain the process of aging [2], but so far none has been universally accepted. However, traditional theories assert that aging is neither a stringent adaptive process nor a strictly genetically programmed process.

According to review by Jin [3], modern biological theories of aging of the human body fall into two basic categories: theories of programmed aging and theories of aging as the result of damage to or errors in the biology of the body. Theories of programmed aging assume that aging is the result of movement along a biological schedule. They are conventionally subdivided into three conceptual subcategories of: (1) The *Programmed Longevity Theory*, in which it is supposed that aging is a consecutive turning on and off of specific genes, and includes a role for genetic instability in the dynamics of the aging process [4]. (2) The *Endocrine Theory*, in which aging is governed by an endogenous biological clock that operating by means of hormones, in which insulin-like growth factor IGF-1 plays a key role. [5]. (3) The *Immune Theory*, which posits that the immune system can be programmed for a decrease in its function that will result in increased susceptibility to infectious diseases and therefore to aging and death [6]. Potentially supporting this is a tangential relationship of imbalances in the immune response to cardiovascular diseases, inflammatory processes, Alzheimer's disease, and cancer [7].

Theories attributing aging to damage or errors in the biology of the body include: (1) The *Wear and Tear Aging Theory*, in which cells and tissues of the body become worn as a result of aging. This theory of aging was first presented by the German biologist August Weismann in 1882, but it still seems quite reasonable. (2) The *Theory of a Vital Activity Level*, according to which a higher basal metabolic rate leads to a shorter life of the organism [8]. The modified version of this theory emphasizes antagonism between growth and stress resistance [9]. (3) The *Theory of Crosslinking*, put forward by Bjorksten (1942) [10], and according to which a

cumulative chemical crosslinking of important macromolecules, such as collagen, will damage cells and tissues and interfere with and delay physical processes, leading to aging [11]. (4) The *Free-Radical Theory*, which assumes that superoxide and other free radicals damage macromolecular components of cells, resulting in cumulative damage to the cell and finally stopping its functioning. A review by Afanasyev shows that reactive oxygen species (ROS) are probably the most important factor in effecting such cellular damage and causing the aging of cells and the organism they constitute, and that an ROS "alarm system" can be considered a development in the free-radical theory of aging [12]. (5) The *Theory of Somatic DNA Damage,* in which aging is considered to be a result of damage to the genetic integrity of cells. It is clear that the DNA of cells in a living organism sustains constant damage; genetic mutations occur and accumulate during aging, causing deterioration in the functioning of the affected cells. And just as damage to the nuclear DNA of eukaryotes and cytoplasmic DNA of prokaryotes can damage their cells, so can mitochondrial DNA lead to mitochondrial dysfunction.

In much the same way, Aubrey de Grey, chairman and director of the SENS Foundation (Strategies for Engineered Negligible Senescence) and editor of *Rejuvenation Research*, emphasizes seven basic types of damage that take place with human aging [13]: (1) mutations of nuclear DNA resulting in cancer; (2) mutations of mitochondrial DNA; (3) the intracellular accumulation of eccrisis, or waste materials; (4) the extracellular accumulation of eccrisis; (5) cell loss; (6) cell senescence; and (7) extracellular crosslinking. Several of the most developed theories and hypotheses for explaining aging are described in the following sections.

2.1 FREE-RADICAL THEORY OF AGING

Among the various theories and hypotheses for explaining aging, the free-radical theory appears to be the most widely accepted plausible explanation for the basic metabolic reactions responsible for aging occurrence [14]. This theory was first formulated in the 1950s. In 1957 Harman advanced the hypothesis that a single general process of accumulation of endogenous oxygen radicals, adversely affecting environmental factors and modifying genetic factors, is ultimately responsible for the aging and death of all living beings [15, 16]. This theory was revised in 1972 [17], when mitochondria were identified as being responsible for most of the free radical reactions connected with the aging process. Harman also assumed that longevity is determined by the intramitochondrial levels of free radicals. This concept holds that oxidative stress increases with increasing age as the result of an imbalance between the generation of free radicals and the body's antioxidant defenses [18], and that an increasing degree of oxidative damage to DNA, proteins, and lipids, and a decreased activity of antioxidant system accompanies the aging process [19, 20]. However, even if it is clear that an accumulation of oxidative damage occurs with increasing age, it is not yet clear whether this process universally promotes aging in all organisms. For example, old and long-living members of the family Drosophilidae have increased levels of the enzyme superoxide dismutase (SOD), which destroys oxidative free radicals, and a corresponding resistance to oxidative stresses [21]. This is also true of long-living mutant worms that exhibit increased SOD and catalase activity with age and a steady resistance to oxidative stresses [22]. And in this latter regard, the possibility of prolonging life in the worm *Caenorhabditis elegans* by means of substances that simulate catalase, SOD, or both, shows that antioxidant substances can play an important role in delaying senescence [23].

The free-radical theory of aging consists of several hypotheses that focus on specific, distinct organelles and types of damaged molecules as key factors in aging [24]. It has, for example, hypothesized that mitochondrial DNA (mtDNA) plays a key role in aging in that mutations in mtDNA can accelerate damage to cells by free radicals, changing the levels of activity of components of the electron-transfer chain in energy metabolism. Malfunctions in electron distribution and accumulation of free radicals would then result in more serious damage to mitochondrial DNA and ultimately prompts mutations in it. In the free-radical theory, this "vicious cycle" of

mutation and free-radical generation is considered the reason of cellular senescence [25]. Yet another hypothesis links aging to an age-dependent decrease in the degradation of oxidized proteins, leading to the intracellular accumulation of damaged and dysfunctional molecules [26]. According to the theory of somatic mutation and aging, the accumulation of genetic mutations (caused to a large extent by oxidative damage) in somatic cells is a definite cause of aging [27].

The identification of free-radical reactions as promoters of the aging process means that interventions aimed at restricting or inhibiting free-radical reactions should reduce the rate of aging and of age-related biological changes and illnesses [28]. An ideal "golden triangle" of oxidative balance has been described [29] in which oxidants, antioxidants, and target biomolecules exist in a balanced equilibrium with one another.

A direct consequence of the free-radical hypothesis has been the clinical use of antioxidants for elderly individuals. However, although increasing attention has been given to the use of antioxidant nutritional supplements in nearly all developed countries, proof of their utility is still poor and ambiguous. Even though some epidemiological research has shown that nutritional supplements containing vitamin E reduce the risk of cancer and cardiovascular disease, such observations are not universal, and there is considerable contradictory information about their utility [30]. Uncertainty about the utility of antioxidants even persists in studies of their effects in experimental animal models [31].

Nonethless, although available information makes it impossible to unconditionally accept or argue in favor of the theory of oxidative stress as the fundamental explanation for aging, an obvious need exists for continuing careful research into the effects of biological oxidants and antioxidants in animal models, as well as for controlled clinical studies of their effects toward a deeper understanding of the role of oxidative stress in aging and longevity [32].

2.2 MITOCHONDRIAL THEORY OF AGING

Currently, the free radical theory and the theory of damage to mtDNA, which seem reasonable explanations for age-associated illnesses, are the two most widely discussed theories of aging, and strong data support the concept that mitochondria play a key role in the pathogenesis of some neurodegenerative diseases.

Basically, the mitochondrial theory of aging represents a special case of the free-radical theory [41, 42]. Mitochondria have their own mechanisms for repairing DNA damage caused by exogenous and endogenous agents, in which free radicals play a frequent role. Although mtDNA represents only from 1% to 3% of the genetic material of animal cells, it makes a proportionally much greater contribution to cellular physiology than might be presumed on the basis of this small percentage. Located near the source of oxidative free radicals, mtDNA is an easy target for these radicals' undesirable effects. As they accumulate, mutations of mtDNA are directly responsible for deficiencies in oxidative phosphorylation, generating active forms of oxygen. Oxidative damage to DNA transforms the base components of its nucleotides and causes other types of damage. Thus, for example, aging is accompanied by an accumulation in DNA of 8-oxoguanine, an oxidation product of the nucleotide guanine, which induces damage to DNA through mismatched base pairing with adenine and substitutions of thymine for guanine and adenine for cytosine in the base-pairing process. The damage to mtDNA is usually much more extensive and longer lasting than the damage to nuclear DNA [33, 34].

The hypothesis that an accumulation of damage to mitochondrial DNA will accelerate aging is based on the greater damage caused by chemical mutagens and lipophilic carcinogens (e.g., polynuclear aromatic hydrocarbons) to mtDNA than to nuclear DNA [43, 44, 45, 46]. Moreover, because mtDNA encodes polypeptides essential to the electron-transport chain, any mutations of mtDNA inevitably influence electron transmission and energy transfer. In turn,

defects in the electron transport chain may have pleiotropic effects by affecting the generation and transfer of cellular energy [35]. The Framingham study of ischemic heart disease and macrobiotics found that longevity is more strongly connected with maternal than with paternal mortality, this circumstance gives some grounds to suspect that mitochondrial DNA, which is transmitted maternally, may play a role in determining life expectancy [36]. Even if this remains debatable [37], some research has clearly shown that life expectancy is linked to particular polymorphisms of mtDNA [38, 39, 40]. The mitochondrial theory of aging is often considered a development and refinement of the free radical theory [41, 42]. As they accumulate, mutations of mtDNA are directly responsible for deficiencies in oxidative phosphorylation, generating active forms of oxygen.

Technologies directed at correcting mitochondrial dysfunction represent a new aspect of antiaging therapy [47]. They are in part founded on the concept that oxidative stress causes damage of mitochondria which is propagated by a vicious cycle in which the damaged mitochondria generate increased quantities of reactive oxygen species (ROS) that in turn progressively worsen the damage to these organelles. In the highly plausible opinion of Romanoetal [48], consideration of aging as a result of oxidative stress indicates that antioxidant technologies directed at mitochondria are reasonable measures for reducing the rate of aging and preventing age-associated pathologies.

2.3 GENETIC THEORY OF AGING

The genetic theory of aging assumes that aging is the result of changes in gene expression [49, 50]. The confirmation that changes in gene expression do control the aging process would represent a great stride forward in understanding the mechanism of aging and provide a starting point for activity directed at retarding aging. Although it is clear that the expression of many genes varies with age, it is improbable that changes in the expression of any specific genes can immediately be linked to the aging process [51]. At present, proof of the legitimacy of a genetic theory of aging is lacking, and aging is typically considered a stochastic process rather than a programmed mechanism directly controlled by genes. Nevertheless, at least 15 genetic manipulations are known that prolong life in organisms such as yeast, fruit flies, nematode worms, and mice [52]. So far, however, the way in which proteins encoded by these genes participate in regulating longevity remains unknown. If and when this does become known, the measures to be taken for rejuvenation on the basis of the genetic theory of aging are likely to involve the reprogramming of cells that are in a pluripotent state.

As noted above the research so far done with model organisms has confirmed a role for genes in influencing of the aging process, as discussed in the following sections.

Yeast. Genetic research on the yeast *Saccharomyces cerevisiae* has been continuing since 1959 [53]. It has shown that the *sir2* (Silent Information Regulator Two) gene extends the lifespan of this yeast by 30% [54] by participating in the processes of response to stress and caloric restriction [55]. The homologue of *sir2* in mammals is the *SIRT1* gene. Mice in which *SIRT1* is switched off are born with obvious delays in physical and mental development. Depending on their genetic background, such mice often die shortly after birth, and the surviving animals are sterile and weaker than controls [56]. Significant changes in life expectancy have not been reported for mice heterozygous for *SIRT1* [57]. An excess of *SIRT1* is observed in mice undergoing caloric restriction [58]. Higher doses of *SIRT1* in murine pancreatic β-cells increase the release of insulin [59]. Mice with a temperate overexpression of *SIRT1* gain weight on the same basis as controls with a high-fat intake [60]. Moderate overexpression of *SIRT1* slows an age-associated increase in hypercardia, apoptosis, and the appearance of biomarkers of aging, while a high level of overexpression of this gene leads to cardiomyopathy and other adverse effects [61].

Nematodes. The nematode *Caenorhabditis elegans* was the first multicellular organism whose genome was completely sequenced and the first organism in which genes governing longevity were discovered. A key finding in *C. elegans* is that of insulin signaling, which has pleiotropic effects on the aging process. The gene designated *age-1* is a recessive allele in *C. elegans* that increases the lifespan of these worms by an average of 40% at temperatures over 20° C and by 65% at temperatures exceeding 25° C. The maximum recorded life extension in nematodes with *age-1* was 110% or 46.2 days. It is most likely that the effect of *age-1* is associated with a depressed self-fertilization function of nematode worms or with other unknown metabolic or physiological changes [62]. The downregulation of insulin signaling significantly prolongs the life of nematode worms [63]. Molecular characterization of the *daf-2* and *age-1* genes of nematode worms showed that *daf-2* is homologous to the mammalian genes that encode the structure of the insulin receptor (IR) and the receptor for insulin-like growth factor-1 (IGF-1R) [64], and that the age-1 peptide encoded by the *age-1* gene is homologous to catalytic subunits of phosphatidylinositol 3-kinase (PI3K) located below the IR and IGF-1R [65]. The downregulation of insulin signaling also prolongs the life of the fruit fly *Drosophila melanogaster* [66], and depression of insulin–IGF-1R signaling influences the lifespan of mice [67]. Another gene, the *daf-12* gene, does not extend the life of nematode worms, but certain combinations of *daf-2* and *daf-12* increase these worms' life expectancy by almost fourfold [68]. The *daf-16* gene is the main target of insulin–IGF-1R signaling. The presence of *daf-16* active proteins contributes to an increase in the lifespan of mutant worms with depressed insulin–IGF-1R signaling [69].

Drosophila. To date, no multicellular organism has had its genetics investigated to the same extent as has *Drosophila melanogaster*, which was the the first organism in which heredity was linked with the aging process [70]. It has been established that overexpression of the *CAT* gene of *D. melanogaster* causes a noticeable delay of aging in a short-lived species [71], but does not cause any changes in long-lived flies [72]. The overexpression of *CAT* also prolongs the life of mice, but this is due to a low frequency of cardiac pathologies rather than to a delay of the aging process [73]. In mammalian cardiomyocytes, the overexpression of *CAT* slows the development of an age-dependent contractile dysfunction [74]. Notwithstanding a frequently cited important role of *CAT* in aging, direct proof of such an effect has not so far been found. Another gene of *D. melanogaster* is *Chico*, which encodes a peptide involved in insulin–IGF-1R signaling. Mutation of *Chico* increases the average life expectancy of flies homozygous for this gene by 48% and that of heterozygotes by 36% [75]. Overexpression of the *sod1* gene in motor neurons of *D. melanogaster* increases the lifespan of affected flies by 40%. Increased resistance to oxidative stress indicates that such an abnormal life expectancy is associated with increased metabolism of RO. These results reveal that the presence of *sod-1* in motor neurons is an important factor affecting the aging process and lifespan of *Drosophila* [76]. Additionally, the overexpression of *sod-2* in *D. melanogaster* has a life-prolonging effect [77]. Abnormalities in the repair processes for cellular constituents such as DNA, proteins, and membranes reduces the life expectancy of various model organisms. Thus, the absence of *mei-41*, a gene involved in DNA repair, decreases the lifespan of *D. melanogaster*, whereas flies with one or two extra copies of this gene live much longer than the average for this species [78]. Overexpression of the *pcmt* gene, which is involved in the repair of proteins, also promotes longevity [79].

Mouse. The mouse, *Mus musculus*, is the short-lived model organism genetically closest to humans, with which it has 79% genetic homology [80]. Almost all of the known genes involved in mouse longevity have homologues in humans [81]. In transgenic mice, the overexpression of *gh*, the gene encoding growth hormone, has different phenotypic effects, including a significant reduction in lifespan that may stem from an early onset of pathological changes in the kidneys. However, such mice have other symptoms of accelerated aging, including astrocytosis, a shortened reproductive period, and an early onset of age-related changes in cognitive function.

A considerably slowed aging process and consequently increased life expectancy in mutants of mice with *gh* deficiency suggests an effect of growth hormone on their life span. Further supporting this hypothesis are field studies of the ratio of body size to longevity in *gh*-transgenic mice [82]. The *Klotho* gene encodes a mammalian hormone that negatively regulates the activity of the insulin receptor and IGF-1R, supressing their autophosphorylation [83]. Genetically reduced expression of this gene leads to the early onset of various age-associated diseases, including ectopic calcification, skin and muscle atrophy, osteopenia, calcification of the aorta, and pulmonary emphysema [84], whereas its overexpression inhibits insulin-IGF-1R signaling, prolonging the life of the model organism [84].

Cellular apoptosis or the arrest of cell division can be results of cell damage. The *p53* gene is a tumor suppressor gene that participates in control of the cell cycle, apoptosis, and DNA repair [85]. Experiments conducted on model organisms suggest a role for *p53* in aging. Studies of *C. elegans*, *D. melanogaster*, and *M. musculus* suggest that inhibition of the expression of *p53* prolongs life [86], although other studies indicate that it increases risk of cancer in mice [87]. Allelic variants and gene mutations of *daf-2* and *FoxO*, the respective homologues of the human *IGF-1* and *FOX1-4* genes can prolong the lives of fruit flies and mice by almost twofold [88]. The *KL-VS* gene, which participates in controlling insulin synthesis through the *IGF-1* gene and in bone turnover through the D-VDR-3 vitamin gene, prolongs the lifespan of mice by up to 30% [89, 90]. Mice and rats show significant prolongations of longevity, to 150% of its average value, with a dwarfism mutation in the *GF* gene for growth hormone that opens an "insuline cascade," and with mutations in the *PROP1* gene that modulates the activity level of pituitary gonadotropins.

Among other genes affecting longevity are the *CAT* gene, which encodes catalase, the enzyme that deactivates peroxide compounds; the *P66Shc* gene, whose product destroys free radicals; and the *Clock*-family genes, which regulate the synthesis and activity of ubiquitin, or coenzyme Q10, which facilitates the degradation of all cell-metabolic toxins by the proteasome of the cell [91]. Some mutations of mitochondrial genes (*C150H*, *517BA*) have also shown a beneficial effect on longevity, reducing the process of cell respiration, and so have mutations in the gene *BCL-2*, whose protein product, Bcl-2, exerts an antiapoptotic effect and increases the stability of the mitochondrial membrane. A mutation in codon 405 of the *CETP* gene, which encodes cholesterol ester transfer protein, leads to an increase in the size of lipoprotein components in the blood that forestalls the formation of atherosclerotic plaque, and has also shown a beneficial effect on longevity [92]. Also noteworthy is the *PPARA* gene, encoding the peroxisome proliferator-activated receptor alpha, which controls the expression of many genes including those involved in fatty acid and glucose metabolism. The polymorphism from guanine (G) to cytosine (C) in codon 327 of this gene resaults in a shift from aerobic glycolysis (genotype GG) to anaerobic glycolysis (genotypes GC or CC) [93].

Genetics of human aging. The most important result of the international Human Genome Project has been the identification of almost all human genes, many of which have been shown to be directly or indirectly involved in the process of aging [94]. Table 2–1 provides a short list of human genes that have been found to be associated with aging in population studies.

An apparently healthy influence of starvation on human longevity may be explicable through an increment in the activity of genes of the sirtuin family. Activity of these genes can be induced through external factors, such as resveratrol in supplemental form or in red wines [97]. In addition to resveratrol, 18 other substances present in plant matter are now known to activate *SIRT* genes, which encode sirtuins. Some of these sustances are now undergoing clinical investigation, and some studies are examining the *SIRT* family of genes as potentially the major family of genes governing human aging, whose regulatory functions not only encompass structural genes but also many genes that exert their own regulatory functions in encoding various transcription factors [97]. Continuing research will show whether the genes of the *SIRT* family do play

Table 2–1. Experimentally determinate and confirmed genes of human aging

No.	*Gene symbol*	*Name/Function*	*Reference No.*
1	FOXO 1-4	Insulin and insulinic growth factor receptor IGF-1	96
2	KLOTHO	Insulin metabolism, IGF1, vitamin D	57
3	PROP-1	Modulation of pituitary hormone levels	95
4	HGF	Human growth hormone	59
5	CLOCK	Synthesis of ubiquitin (coenzyme Q10)	95
6	CAT	Catalase (deactivation of peroxide compounds)	58
7	P66She	Deactivation of free radicals	66
8	MTP	Microsomalic carrier protein	65
9	CETP	Protein-carrier of cholesterin	65
10	TOR	Growth and cellular nutrition	96
11	PPARA	Regulator of fatty acid metabolism and glycolysis	65
12	SIRT-1	Supposed main regulator of aging	60, 61

a dominant role in aging or whether they are important but not uniquely controlling in this highly complex multilevel process. Of particular related interest are data showing a possibly oncogenic role of the *SIRT 1* gene [92]. Although this may seem surprising, a link between oncogenicity and longevity has been noted for a range of other genes, including *p53* and the lethal giant larvae or *lgl* gene of the fruit fly, both of which act as tumor-suppressor genes. [98], and the *FOXO* gene mentioned earlier [88]. Heterozygosity for these genes blocks tumor progression, while homozygosity accelerates aging through increased apoptosis and the rapid depletion of stem cells. With regard to the problem of aging, it potentially noteworthy that the activation of one recently identified nanog gene, leads to a sharp rejuvenation of human and other mammalan cells and induces their regressive transformation to stem cells, offering promise for the direct recovery of damaged organs and tissues [99].

Some of the regulatory genes involved in aging have already been identified. Investigation of their mechanisms of action and the quest for other aging-related genes must continue. Nevertheless, it is essential to recall that most of the genes with known or possible roles in aging are still undergoing investigation and cannot be considered as providing any basis for mass prognostic screening in relation to aging or age-related diseases. Nor do allelic variants of these genes show causal linkages to the many illnesses that influence longevity. In sum, it can be stated that the role of the genome in aging must first continue to be identified through the work of geneticists and then become the ground for developmental science.

In this effort, the input of individual genes, gene families and the whole genome can be objectively evaluated by careful analysis of expression and effect of each on the different stages of development in the normal state and in abnormalities. Such investigation was begun only recently, but already its results deserve a careful study [100–102]. The peculiarities of the expression profiles of almost all of the 33,000 human genes in 74 individuals ranging from 27 to 92 years of age were studied with the use of gene-expression chips [100]. The genes were derived from healthy sites in kidneys that were removed because of different medical conditions. Changes in expression were noted in 985 genes, in 742 of which the change was an increase in gene expression and in 343 of which it was a significant reduction in expression. These genes were called "age-regulated genes" (ARG). The age-related expression profiles of these ARG corresponded to anatomical and physiological changes in the kidney from which the genes were obtained. Of interest was a partial overlap in the expression profiles of genes in the renal cortex and those in the renal medulla. An analogous age dependence of gene expression was noted in a comparison of the expression profiles of genes in different organs. Thus, among 447 ARG found in the kidney, 227 were also found in other tissues, and their expression also changed

with increasing age. It is notably, the expression of all of these ARG works in one direction. On this basis it was concluded that aging is linked not only with the entire genome but also with individual genes, the array of which can vary in different tissues. Weakening of these genes' activity should lead to single specific disorders in cell function with aging, and finally to a decline in the specific functions of whole organs. The significance of this is that aging is accompanied by a slow decrement in transcriptional activity and by functional degeneration of the entire genome.

The concept of retarding aging through the gene known as *mTOR*, for "mammalian target of rapamycin," so named because it is inhibited by the immunosuppressant drug rapamycin, or sirolimus, is based on regulating the aging process by targeting the mTOR protein encoded by the gene, which is a serine/threonine kinase that regulates cell growth, proliferation, and survival, gene transcription, and other cellular functions. Among the functions of mTOR are the integration of signals engendered by insulin, insulin-like growth factors (IGF), and various amino acids, and monitoring of the cellular nutrient and energy levels and the reduction-oxidation state of the cell. In cell culture [107, 108], as well as in rodents and humans [109–112], nutrient materials activate mTOR and cause cellular insulin-resistance. The activation of mTOR blocks its sensitivity to insulin, creating insulin resistance [107, 113], which is associated with some cases of early menopause [114]. Most of the genes related to aging and longevity are to some degree influenced by mTOR [103, 104], which is essential during embryonal development [105, 106] and whose regulation becomes aberrant in various cancers and other diseases.

2.4 TELOMERIC THEORY OF AGING

The cellular theory of aging was introduced in 1965, when the aging of cells was described as a process characterized by a limited number of cell divisions [115]. This restriction of cellular "replicative potential" occurs after a number of cell divisions and inevitably results in irreversible changes of physiology [116]. Telomeres are specialized, repetitive sequences of DNA at the ends of eukaryotic chromosomes. Human telomeres consist of repetitive sequences two thymines, adenine, and three guanine nucleotides (TTAGGG) [117], and are protected against excessive degradation by the enzyme telomerase, a ribonucleoprotein that acts as a reverse transcriptase supporting lengths of chromosomes [118]. The key function of telomeres appears to be the protection of genes near the ends of chromosomes from damage during cell replication. In actively dividing cells, some quantity of DNA at the end of the chromosome is lost with each cell division. This process of progressive shortening of the chromosomal telomeres begins soon after fertilization, when the cells of the zygote begin dividing. As a result, the telomeres at the ends of chromosomes become increasingly short and this finally results in the end of cell proliferation [121], whereas immortal cells maintained in culture have telomeres of stable length. In specific types of immortal cells, such as stem cells, germ cells, and T-cells, telomerase consistently supports the length of the telomere. The absence of telomerase can be a basis for cellular aging [119], and telomerase also provides a consistency of telomere length in cancer cells, permitting them to avoid replicative senescence [120].

Consequently, there is a link between the availability of telomerase, chromosomal consistency, and cell mortality, and the shortening of telomeres and loss of telomerase in typical somatic cells appear to be involved in what might be described as a "molecular clock" that initiates cellular aging [122] and governs the proliferative capacity of cells and occurrence of age-dependent pathology [123–125].

Because telomeres and telomerase are connected with cellular aging and apoptosis, it is logical to assume that they play key roles in aging and the onset of malignancies and hereditary syndromes, as well as in the development of chronic age-associated illnesses [129]. This theory has established new trends for research on interventive measures that can retard

aging [126, 127]. Unfortunately, much remains unknown about the mechanisms controlling the expression of telomerase in somatic cells, and it is not yet clear how this knowledge could improve the understanding of human aging. Although the results of intensive research show that telomerase can be closely involved in cellular aging and that its control may hold great promise, the understanding of these age-related mechanisms is still in its beginning stages. Thus, for example, telomeric dysfunction has been suggested as activating the mechanism of cellular aging and apoptosis mediated by *r53*, a gene involved in the synthesis of adenosine triphosphate (ATP) [128].

2.5 THE INFLAMMATORY HYPOTHESIS FOR AGING

The importance of inflammation in the course of aging has only been recognized fairly recently [130, 131]. Nevertheless, hypotheses for its role in aging has developed rapidly, and as emphazised in a review by Pizza et al. [134], many studies have shown that most of the phenotypic characteristics observed in the course of aging are the result of a chronic inflammatory condition named "inflammaging" [132], which is under some degree of genetic control. Even if a link between inflammation and aging is disputable, however, it is important to emphasize that a theory relating the two complies with other theories of aging. As an example of this, a close association is recognized between inflammation and oxidative damage [133].

2.6 IMMUNE THEORY OF AGING

In 1989 Franceschi advanced his immune or network theory of aging [135], in which he assumed that aging is indirectly controlled by a network of cellular and molecular immune mechanisms. In particular, this considers macrophages as basic modulators of a vicious cycle, of nonspecific immunity, inflammation, and stress. The theory maintains that the activation of macrophages by chronic stress can explain a pattern of chronic, subclinical inflammatory processes in the elderly, and that lymphocytes are also affected by continuous age-related antigenic stress. A result of this is a decrease and even possible exhaustion of the pool of naive immune cells and T-lymphocytes. This hypothesis for a role of the immune system in aging by the data about increase of disease by tumors and the big susceptibility to infections of older persons is supported [136, 137].

Although the exact mechanisms responsible for age-dependent immunosenescence are unknown, a number of hypotheses have been advanced to explain this. A link between structural and functional immunosenescence and a reduction in the generation of naive T-lymphocytes is a basic, fundamental reason for inferring a role of the immune system in aging [138–140]. This decline in naive T-cells weakens the ability of the immune system to adaptat and respond to new antigenic stimuli. Another reason for inferring a role of the immune system in aging is a loss with aging of various growth factors and hormones influencing lymphoid structures and promoting thymic function. For example, it is known that growth hormone (GH), insulin-like growth factor-1 (IGF1), keratinocyte growth factor (KGF), nerve growth factor (NGF), interleukin-7 (IL-7), and gonadotropic hormone releasing factor (GnRH) all influence immunosenescence [141–144]. The most extensive studies done with GH [145, 146] and ghrelin (stimulator of growth hormone exhaust and regulator of eating behavior) in this regard [147, 148] demonstrate their contribution to thymic functioning and the immunosenescence of such functioning. Such studies have made it possible to consider the of use of hormone combinations (GH + GRL), cytokines (IL-7), and growth factors (IGF-1) for inhibiting aging of the thymus and immune system in elderly persons [149].

2.7 NEUROENDOCRINE THEORY OF AGING

Recent concepts of two-way communication between the nervous and immune systems have increased [150]. With aging, there is a decline in function of both the immune and nervous systems as well as increasing dysregulation of their relationship, resulting in a progressive loss of homeostasis and increased risk of death [151, 152]. The neuroendocrine theory of aging assumes that it results from changes in neurologic and endocrinologic functions that are crucial to for homeostasis. Aging-related changes not only selectively affect neurons and hormones controlling evolutionarily significant functions, such as reproduction, growth, and development, but also influence the degree of adaptation to stress. Thus, longevity is controlled by "internal clocks." Changes in the working of these clocks, such as decreases in their reactions to stimuli or excessive or insufficient coordination, lead to aging [153–155]. A major component of this theory is that the hypothalamo-pituitary-adrenal axis is the basic regulator of the beginning and end of each stage of life and the major factor in preserving and maintaining internal homeostasis despite continuing change in the environment [156]. According to this theory, aging should be considered to result from a decrease in the ability to endure stress. With aging, there is a decrease in the regulatory function of the sympathetic division of the nervous system characterized by: (1) reduction in the numbers of catecholamine receptors in peripheral tissues; (2) decrease in the level of Heat shock proteins, which normally increase stress-tolerance; (3) a decrease in the catecholamineergic stimulation of heat shock proteins; (4) a reduction in the levels of circulating GH, testosterone, estrogen, dehydroepiandrosterone, and other hormones. Although interventions directed at retarding aging according to neuroendocrine theory affect some of its physiologic effects, too often they have adverse side effects. Further research is needed before GH or other endocrinologic agents for retarding aging can be considered safe and useful on a long-term basis.

2.8 THE THEORY OF "CROSSLINKING" OR GLYCOSYLATION OF PROTEINS

According to the theory of "crosslinking" or protein glycation as a basis for aging, the aging mechanism is somewhat similar to the effects of free-radical activity. Proteins can be damaged both by free radicals and through glycosylation (glycation, the Maillard reaction, nonenzymatic glycosylation). In this reaction, a reduced sugar forms a direct chemical bond with a protein, without the catalytic activity of an enzyme. The glycosylation occurs at the amino groups of lysine and arginine, which are involved in peptide bond formation [157]. The result is a ketoamine, named an "Amadori product" [158]. In this theory, sugars are given the role of aggressive substances, with the primary role given to glucose, which is always present in an organism. Glycosylation and the formation of Amadori products are reversible reactions, whereas the oxidation of an Amadori product, resulting in the formation of advanced glycation end products (AGEs), is an irreversible process [159]. It is at this point that the theory of glycosylation of proteins intersects the free-radical theory, since the formation of AGEs increases the cellular content of free radicals by 50-fold. The formation of AGEs resulting in the crosslinking in collagen the walls of blood vessels is involved in the initiation of atherosclerosis and of nephropathy in diabetes, as well as in the formation of cataracts and in Alzheimer's disease [160–162]. The formation of AGEs is a general indicator of the aging of skin, muscles, the lungs, blood vessels, and other organs and structures [163].

Recently, Naila Rabbani and Paul Thornalley of the Clinical Sciences Research Institute of the University of Warwick, England, who are involved in the study protein glycosylation and its role in cellular damage and aging reported that protection of mictochondrial proteins against glycation by the endogenous dicarboxylic acid products known as methylglyoxal and glyoxal,

known structurally as dicarbonyls, prevents an increase in free radical generation and the action of oxidative stress on the proteome during the aging of nematodes. This indicates that damage from glycosylation of the mitochondrial proteome results in functional damage to mitochondria that in turn results in oxidative stress.

The theory of glycosylation of proteins as a cause of aging is a special case of the theory of damage to proteins as the cause of aging and the most widely held such case. It is the basis for seeking and creating products that break crosslinking and transform the products of this into nutrients for the cell. Extensive studies have been done of biologically active substances that suppress the glycosylation of proteins, such as studies of vitamin B6, one of its vitamers its pyridoxamine, salidroside, and ginseng extracts [164–166].

Each of the basic theories of aging, including the free-radical theory, immune theory, inflammatory hypothesis, and mitochondrial theory, contains useful and important information for understanding the physiological changes occurring with aging. Nevertheless, none of these theories yields encompassing explanations for the process of aging [167]. On the basis, the quest for a single reason for aging, such as the deletion of a single particular gene, has recently given way to the view of aging as an extremely complex multifactorial process [168–170]. In actuality, however, it is logical and very probable that several processes interact and work at different levels of functional organization to impel the aging process [171]. Clearly it is more productive to consider that the various theories of aging complement rather than cancel each other, since this permits a more or less logical explanation for most of the process of normal aging [172, 173]. The following sections discuss some molecular mechanisms that play a role in the various theories described above and which are important for the pathogenesis of aging.

2.9 MOLECULAR MECHANISMS OF AGING

It is clear that regulation of the process of cellular aging varies considerably depending on the organism being studied but also on type of cell in the organism [174]. Thus, for example, telomeric shortening is the major reason for the aging of human fibroblasts [175], whereas in the fibroblasts of mice aging depends not on the shortening of telomeres but on oxidative stress [176, 177].

Various stimuli promoting aging (eg, oxidative stress, telomeric shortening) exert their effects through genetic pathways. In particular a role for the *p53* gene in aging is now often discussed, and it has even been suggested that the *p53* gene can act to stop aging [178–182].

The *p53* gene is known as a key tumor-suppressor gene [183–186]. However, it has been determined that *p53* exerts a huge influence on the longevity of different organisms through this tumor-suppressor effect. In preventing the development of tumors early in life, *p53* contributes to longevity [187, 188], and recent research has shown that *p53* also plays an essential role as a regulator of aging in worms, drosophilidae, mice, and humans. Interestingly, new data confirm the assumption that *p53* can control aging independently of its tumor-suppressive function [189]. Possibly related to this is the knowledge that in senescent cells, *p53* is phosphorylated and its level of transactivation increases although the levels of mRNA and protein encoded by the gene remain invariable [190–192].

An influence of p53 on human aging and longevity has been found in several epidemiological studies [193–196]. Most interestingly, in a prospective study of individuals age 85 or older (n = 1226), individuals homozygous for the p53 P72 allele exhibited a significant 41% enhanced survival compared with individuals with at least one p53 R72 allele (P = 0.032), although they had a 2.5-fold increased cancer incidence (P = 0.007) [197, 198]. A study conducted by Smetannikova et al. [199] found that the P72 allele of *p53* was present in 131 long-lived persons in the Novosibirsk and Tyumen Oblasts of the Russian Federation. More recently, Ørsted et al. [200] investigated the influence of a single nucleotide polymorphism (SNP) in codon 72

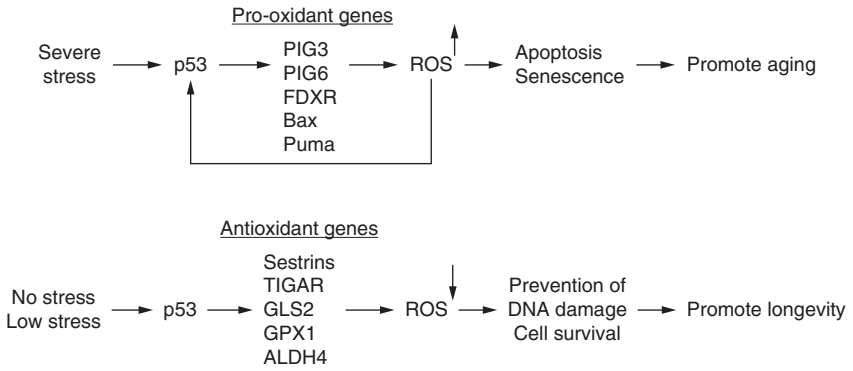

Figure 2–1. *p53* as a regulator of oxidative stress and the products of reactive oxygen species (ROS), and the effects on longevity of modifying the effects of ROS.

of the *p53* gene on longevity in a cohort of 9219 Danish subjects aged 20 to 95 years. They found found a significantly greater 12-year survival rate, of 3%, in persons with a single P72 allele ($p = 0.003$) and a 6% greater than average rate of survival in subjects homozygous for the P72 allele ($p = 0.002$), than in subjects homozygous for the R72 allele of *p53*. The median life expectancy of subjects homozygous for the P72 allele was 3 years greater than for those homozygous for the R72 allele. The same investigators also found a greater rate of survival after the development of cancer among homozygotes for the P72 allele of *p53* than for those homozygous for the R72 allele.

These clinical findings, as well as the results of other research, indicate that although its role is complex and depends on a great number of factors, *p53* participates in the regulation of aging and longevity.

The mechanisms by which *p53* regulates aging and longevity remain unclear. Some suggested mechanisms include regulation through insulin/IGF-1, TOR, and stem cells, and effects on processes of oxidative stress and the synthesis of reactive oxygen species (ROS). In this regard, it is thought that *p53* exerts negative transcriptional control on seven target genes that regulate insulin/IGF-1 and TOR signaling, thereby acting to suppress cell division and growth. At the same time as it extends longevity by suppressing insulin/IGF-1 and TOR signaling, p53 control aging and longevity through a suppression of signaling along these two critical pathways [201–206]. Beyond this, *p53* shows increased activity and plays an important role in stress-induced forms of senility [207–211].

A schematic diagram provided by Feng et al. [212] and shown in Figure 2–1 illustrates the prospective role of *p53* in regulating the aging process through effects on oxidative stress.

The figure indicates that *p53* stimulates either pro- or antioxidant activity according to the type and degree of stress signaling. In non-stress situations or at low levels of stress, *p53* selectively induces the expression of a group of antioxidant genes, such as those encoding the sestrins, TIGAR, GLS2, GPX1, and ALDH4, with the effect of decreasing the intracellular ROS production. This antioxidant function of *p53* protects cells from oxidative stress and ROS-induced damage to DNA, and allows greater cell survival, thereby preventing aging. In response to intensive stress, *p53* selectively stimulates the expression of various prooxidant genes including *PIG3*, *PIG6*, *FDRX*, *Bax*, and *Puma*, increasing the cellular generation of ROS. This prooxidant function of *p53* results in *p53*-mediated apoptosis and aging.

Research has also shown that changes in the activity of *p53* influence the proliferation, quantity, self-renewal, and differentiation of stem/progenitor cells [213–215], and this effect may also be

considered a mechanism by which *p53* influences on aging and longevity. The studies of worms, flies, mice and humans described earlier show convincingly that *p53* is a highly important if complex component in both promoting and limiting aging and longevity. Its contribution to longevity by preventing tumor development early in life was described earlier, but as also noted, its role in regulating aging and life expectancy independent of its tumour-suppressing function are still not clear. Only further research will permit an understanding of its complex role in these other processes.

The following sections provide generalized descriptions of methylation, glycosylation and oxidation as basic biochemical processes developing in the course of aging.

Methylation of DNA and Aging

Molecular events determining gene transcription have a fundamental interest for gerontologists, since the regulation of gene expression radically influences aging and the biological changes that accompany it. As indicated in earlier sections of this chapter, factors influencing gene expression without directly causing changes in the genetic code clearly play a role in aging. One of these is the methylation of DNA [216]. Up to 5% of all cytosine residues in the DNA of mammals are methylated at the 5′ position, with the formation of 5-methylcytosine (5mC). This is a unique method of modifying the DNA of higher eukaryotes. Methylation of cytosine occurs symmetrically in both strands of diploid DNA, and residues of 5mC are always paired with residues of guanine as a result of 3′-tailing. Methylated residues of cytosine implement various functions, but even more important is the involvement of DNA methylation in regulating gene activity. Changes in methylation, and particularly the demethylation of dinucleotides in vertebrates, is associated with changes in levels of transcription. An age-related demethylation of DNA was initially described in 1973 by Vanjushin and co-workers [217]. They found, differences in the degree of demethylation in rat tissues, in which methylation was more prominent in brain tissue than in liver tissue. Subsequently, an age-related depression of 5mC was found in the lungs and cultures of skin fibroblasts, in the latter of which demethylation was associated with a reduced capacity for cell growth in culture [218]. It has also been suggested that age-related demethylation predisposes cells to malignant transformation.

Table lists a number of human genes with aging-related effects caused by hypermethylation [219].

Gene	*Function*	*Chromosome and Location*	*Organ*
CSPG2	Chondroitin sulfate proteoglycan	5q12-14	Large bowel
DBCCR1	Candidate tumor suppressor	9q32-33	Bladder
ER	Estrogen receptor	6q25.1	Large bowel
HICI	Zinc-containing protein	17p13.3	Prostate
IGF-2	Insulin-like growth factor-2	11p15.5	Large bowel
MYODI	Myogenesis factor	11p15.4	Large bowel
hMLH1	DNA repair	2q22	Stomach
N33	Candidate suppressor of prostate cancer	8p22	Large bowel

It has been found that aberrant regions of DNA methylation ranging from 0.5 to several thousand base pairs are essential to the inactivation of gene activity and are often observed in cancer cells. These areas are observed near genes, and are often located next to the promoter regions of widely expressed genes. Age-related hypermethylation was observed in the physiological mucous coat of the large bowel and in a number of other organs, and chronic inflammatory processes such as chronic ulcerative colitis or infection with *Helicobacter pylori* are associated

with excessive methylation. It was also observed that age-related methylation increases linearly with age, although the degree of its increase may vary. The methylation of a number of genes involved in DNA repair, such as hMLH1, MGMT, and GSTP1, leading to their inactivation, can promote an accumulation of mutations with aging and probably accelerates aging and increases the risk of cancer.

Glycosylation of Proteins and DNA

Nucleic acids and proteins can be modified through the addition of sugars to their free amino groups, which leads to structural and functional reorganizations of their molecules. Interest in the Maillard reaction between glucose and proteins grew considerably after it became obvious that through covalent bonding, glucose by itself, without the participation of enzymes, can modify proteins in vivo [220]. The nonenzymatic glycosylation of proteins includes the bonding of glucose to free amino groups with the formation of Schiff bases and their subsequent transformation into stable Amadori products and then into advanced glycosylation end products (AGE). End products of the Maillard reaction are poorly soluble, resistant to proteolytic cleavage, at least moderately active chemically, and capable of forming intramolecular linkages (eg, in collagen). They can bond covalently to proteins which owing to rapid regeneration (eg, LDL, IgG) and to some other substances having free amino groups (eg, DNA, some lipids), and can chemically inactivate nitric oxide (NO).

The group of the membrane proteins belonging to the superfamily of immunoglobulins which execute function of receptors for deeply glycated molecules has been detected. Receptors for AGE are found on fibroblasts, T-lymphocytes, in mesangial cells of the kidneys, in the endothelium and smooth muscle cells of the vascular wall, and in the brain, liver, and spleen where they are detected in great quantities in macrophages-rich tissues. Macrophages exhibit the most intensive destruction of products of the Maillard reaction, through endocytosis and the synthesis of many regulatory molecules. Some of these, particularly IGF-1 and platelet-derived growth factor (PDGF), stimulate division of fibroblasts, smooth muscle and mesangial cells [221].

The nonenzymic glycosylation of biologically important molecules has become an increasingly important sphere in research on diabetes and the process of normal aging. Monosugars such as D-glucose and D-galactose can themselves or through metabolites bond covalently to protein molecules and bind various proteins to themselves. Aged and diabetic persons were found to show an increased glycosylation of collagen, which contains a considerable quantity of glucose, as compared with normal individuals. Such increased glycosylation of collagen reduces its elasticity, and may explain thickening of the glomerular basal membranes and mesangial matrices of the kidneys and renal failure in diabetes, as well as an age-related depression in renal function. This mechanism is also thought to play a role in arteriostenosis, the reduction of vascular blood flow, and the loss of flexibility of tendons.

Hyperglycemia results in the formation of glycation end products and ROS [222]. It has also been found to result in an accumulation of deletions in mtDNA and to other mutations in cells of the muscular layer of blood vessels. In untreated diabetes with high levels of glucose is accompanied by many signs of accelerated aging, such as inadequate wound repair, cataracts, vascular and capillary damage, and an increased risk of cancer. Accumulation of pentosidine, considered a marker of AGE, is accelerated at diabetes and is considered a valid marker of aging [223]. It has been found that in the dermal collagen of short- and long-lived animals, pentosidine glycation was inversely proportional to specific maximum longevity. The key role of insulin as factor governing longevity has been convincingly shown in various types of invertebrates [224].

As is known, an effective way of retarding of aging is caloric restriction. A possible mechanism for this is a reduction in the concentration of glucose in the blood and of the nonenzymatic bonding of glucose to long-lasting proteins such as hemoglobin [225, 226]. A reduction in the

concentration of blood glucose reduces both the glycosylation of proteins and the peroxidation of lipids. The primary adverse effect of glycosylation is not glucose connection to oxidative long-lasting proteins by free radicals. Nucleotides and DNA also are exposed to nonenzymatic glycosylation, which leads to mutations both through direct damage to DNA and the inactivation of systems for DNA repair and errors of recombination. Such glycosylation also causes chromosome fragility. Studies are being conducted of means for preventing the glycosylation of long-lasting proteins by pharmacological and genetic means. In a related area of work, aminoguanidine may be useful for treating complications of aging and diabetes, through its ability to prevent glycosylation-related changes in basal membranes, atherosclerosis, and renal effects of diabetes. The long term injection of antidiabetic biguanides in mice and rats was found to retard aging of the genital system and to prolong the animals' longevity [227–230].

Even though we mentioned many theories of aging, it is clear that most of them have only historical value. Historically, only two major explanations have been given for aging: according to one of them, aging is the result of a genetic program; according to the other, it is the result of metabolic and structural damage and errors that are bound to occur in any complex organism, such as through the effects of ROS. Because the genetic mechanisms of aging still remain largely unknown, and because the complete elimination of metabolic and structural damage to biological systems is widely considered inachievable, reducing the generation and effects of ROS and other detrimental factors would seem to be the only reasonable means of retarding aging.

The dominant viewpoint of modern gerontology is that the primary reasons for aging have a molecular basis. Thus, for example, most of the papers presented at the 2010 congress of the International Association of Biomedical Gerontology involved the free radical theory of aging. On such basis it is already possible to establish theories sufficient to make aging a biologically understandable process and to work toward means of retarding it.

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Chapter III

Biomarkers and Indicators of Aging: Markers of Biological Age, and Predictors of Longevity

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3.1 INTEGRAL BIOMARKERS OF AGING

If the aging process is defined as a gradual decrease in the efficiency of the physiological regulation of complex multifactorial processes, the individual genotype will clearly impact the rate of aging. Yet to date, markers of this rate remain unclear. Although attempts to define markers that could help to predict this rate of aging have been made for the past two decades, no such markers have been clearly identified.

To characterize the rate of aging of an organism, it is important to determine its biological age. If this biological age is considered to be the degree of age-related change in specific biological features of the organism at every stage of its ontogenesis or development, it can be defined as the remaining duration of life or the probability of death within specified period.

The concept of biomarkers of aging is usually limited to identifiable factors or constants, registering a real biological age, that determine the rate of aging and permit evaluation the effectiveness of any intervention in the aging process. Modifying the definition adopted in the gerontological literature, which is based on the work of Baker and Sprott [1], we propose that a biomarker of aging be defined as a quantitative or qualitative biological parameter or set of parameters that predict the status and function of the body within a framework of time, and permit assessment of the probable effects of remedial technologies and interventions.

Along with determination of the integrated biological age of the organism as a whole is the proposal for determining the biological age of its nervous, cardiovascular, respiratory, excretory, and other systems and even the biological age of its cells. This sometimes also calls for

distinguishing the psychological, intellectual, and social age of an individual human. In the case of physiological aging, the biological and calendar age of an individual are considered the same, whereas the degree of aging, whether accelerated or decelerated, is reflected by the difference in the biological and calendar age of the individual. Premature aging can be attributed to genetic (endogenous) factors, such as mutations in some genes in syndromes of progeria; to external (exogenous) factors such as chemical, toxic, and carcinogenic substances, ionizing radiation, electromagnetic fields, heavy physical labor; environmental factors (pollution); and to damaging habits such as the excessive use of alcohol and use of tobacco. Besides the biomarkers discussed above, it would be desirable to have common biomarkers of aging related to these factors.

Several authors have discussed the criteria that should be satisfied by biomarkers of aging. Thus, according to Arking [2], such biomarkers should:

a. Change with age at a rate that reflects the rate of aging.
b. Reflect the physiological age of the individual.
c. Allow the continuous monitoring of changes in an important bodily process.
d. Be a factor essential for health.
e. Serve as a predictor of life expectancy.
f. Serve as a retrospective marker of aging.
g. Be readily and replicably measurable.
h. Reflect changes that occur during a relatively short time.
i. Be measurable in different animal species.
j. Be non-lethally measurable, non-invasive, and minimally traumatic.

Another set of criteria for biomarkers of aging comes from the Gerontological Research Center of the U.S. National Institute of Aging [3]. These state that such biomarkers should be:

1. Nonlethal.
2. Easily and reproducibly measurable.
3. Readily and rapidly reflect aberrations in aging.
4. Be factors essential for the maintenance of effective health and prevention of disease.
5. Show substantial stability in their reflection of individual differences.
6. Reflect a measurable parameter that can be predicted at a later age.
7. Show significant longitudinal changes with aging that are consistent with data obtained in cross-sectional studies.
8. Reflect fundamental biological processes of aging and metabolism.
9. Be highly reproducible in cross-comparisons of organisms of different species.
10. Show age-related change on a scale that is proportional to the difference in life expectancy for different species.

Both of the foregoing sets of criteria make clear the difficulty in choosing an index biomarker of aging that satisfies all of the proposed criteria in each set. A simpler and more clear set of criteria for biomarkers of aging has been elaborated by the American Federation of Aging Research [4]. These hold that biomarkers of aging should:

1. Predict an individual's rate of aging and be better predictors of longevity than chronological age.
2. Permit monitoring of the basic processes that underlie aging, but not be manifestations or consequences of disease.
3. Allow repeated measurement without harm to humans.
4. Be reproducible in humans as well as in conventionally used laboratory animals.

Data available in the literature allow the formulation of a clear set of requirements that biomarkers used to determine biological age must meet, as follows:

1. Provide information about the functional condition of the body, its metabolic and regulatory systems.
2. Have quantitative characteristics that correlate with age.
3. Be reproducible, sensitive, and specific.
4. Be suitable for use in humans as well as in laboratory animals.

Moreover, in considering specific biomarkers of aging, each biomarker is likely to have both advantages and limitations, as emphasized by McClearn [5].

In an early discussion on the issue of biomarkers, Dilman [6] suggested that there are no age-specific or age-related norms, but there is an ideal (optimal) norm inherent to each individual that is attained at the age of 20–25 over successive 20- to 25-year periods or phases of life, and suggested five parameters for defining the biological age of an individual:

1. Body weight or more precisely, body fat, which can be calculated indirectly in terms of height, weight, and the thickness of skin and fat folds (eg, the triceps skinfold).
2. Blood levels of pre-β- and β-lipoproteins and triglycerides.
3. The serum level of cholesterol and α-cholesterol (cholesterol in high-density lipoprotein [HDL]).
4. The preprandial blood glucose level and the blood glucose level at 2 hours after the ingestion of a 100 g bolus of glucose.
5. The systolic and diastolic blood pressures.

The American Federation of Aging Research (AFAR) uses biomarkers of aging essentially similar to these, based on the their expected predictability in an age-dependent manner. To avoid disturbing the patient and affecting the results of biomarker measurements, such measurements should be simple and inexpensive to make. Table 3–1 [7] presents these integrated biomarkers of aging.

Many attempts have been made to use the body mass index (BMI) as a marker of aging. Long-term clinical trials in patients over 21 years old have led to the conclusion that the total body potassium (TBK) content in the age range of 20 to 30 years (as a reliable proxy to the body's free fat mass index), and that the value of the body cell mass (BCM) allows prediction of the body mass index (BMI) after the age of 60 years [8].

The blood level of erythropoietin (EPO) has been proposed as a predictor of mortality of elderly persons. In the Leiden study, a prospective study involving 599 people aged 85 years and over, the blood level of EPO showed a highly reliable inverse relationship with mortality [9].

Because diseases of the cardiovascular system have historically been the number one cause of early death, it is important to determine as precisely as possible the biological age of the cardiovascular system. This is calculated as follows:

$$PBA_{CVS} = (CA_{ind} - CA_{mean}) \bullet R^2 + CA_{mean}$$

where PBA_{CVS} is the corresponding biological age of cardiovascular system; CA_{ind} is the calendar age of the individual in whom the biological age of the cardiovascular system is being measured; CA_{mean} is the the average calendar age of the examinees; R is the multiple correlation coefficient of the biological age (BA) of the cardiovascular system, including the measured value of the systolic blood pressure (SBP).

$$BA_{CVS} \text{ for men} = 0.19 \bullet SBP + 2.98 \bullet TCI - 7{,}23 \bullet CI + 26{,}45$$

$$BA_{CVS} \text{ for women} = 0.38 \bullet SBP + 3.07 \bullet TCI - 4{,}77 \bullet CI - 1{,}24 \bullet CI$$

Table 3–1. Biomarkers on Routine Physical Examination

Biomarker	*Description*	*Pathologies Associated with Biomarker*
Systolic blood pressure (SBP)	Index of cardiovascular function as maximal arterial pressure with cardiac contractile pumping of blood throughout body	Cardiovascular disease, stroke, coronary heart disease, mortality
Diastolic blood pressure (DBP)	Index of cardiovascular function as minimal arterial pressure with the heart at rest	Cardiovascular disease, stroke, coronary heart disease, mortality
Heart rate (HR) at rest	Index of cardiac activity and overall physical condition	Cardiovascular disease, mortality
Total cholesterol	Indicator of bile acid and steroid hormone status	Indicator in middle age of coronary heart disease and general risk of mortality. Indicator in old age of risk of mortality according to a U-shaped curve of cholesterol concentration versus mortality risk
Low-density lipoprotein	Transportation of cholesterol from liver to tissue cells	Cardiovascular disease, arteriosclerosis, stroke, peripheral vascular disease
High-density lipoproteins	Beneficial carriers of cholesterol	Increasing concentration correlated with decreasing risk of atherosclerosis
Fat	Energy reserve	Myocardial infarction, coronary heart disease, pancreatitis
Blood glucose (fasting)	Measures of blood sugar levels and indicator of risk/status of diabetes	Diabetes, cardiovascular disease, mortality, cognitive impairment
Body mass index (BMI)	Indicator of balance of nutrient intake and energy expenditure	Cardiovascular disease, diabetes, stroke, mortality, some cancers, osteoarthritis
Correlation of waist-hip	Index of abdominal obesity	Hypertension, coronary heart disease, insulin-dependent diabetes, stroke
T-lymphocytes	White blood cells that conduct surveillance against neoplasia and protect against infectious pathogens	Cancer, mortality, atherosclerosis, Alzheimer's disease
Cortisol	Steroid hormone reflecting the reaction to physiological stress	Cardiovascular disease, impaired gluconeogenesis and energy metabolism, impaired immune function, mortality
Electrocardiogram (ECG)	Measurement of cardiac electrical function	Cardiovascular disease, stroke, mortality

where the numerical values are constants of the regression equation; BA_{CVS} is the the biological age of the cardiovascular system in years; SBP is the systolic blood pressure in mmHg, TCI is the measured value of the total conjunctival index, which is calculated as the sum of partial indices that describe extravascular, vascular, and intravascular microcirculation in the bulbar conjunctiva of the eye as determined with a slit lamp, CI is the cardiac index in L/m^2, which is the index of efficiency of the heart, measured either as the ratio of the cardiac output (CO) to the body surface area (BSA) or as the product of the stroke volume (SV) multiplied by the heart rate (HR) divided by the BSA ([SV • HR]/BSA).

Systolic blood pressure (SBP) in mmHg is measured according to the usual method, with the Riva Rocci apparatus on the upper right arm of the subject in the sitting position. The measurement repeated three times at intervals of 5 minutes, and the reading in which the blood pressure is lowest is the one that is used. The stroke index (SI) is measured as the ratio of the cardiac stroke volume to the BSA of the subject, and reflects the association between myocardial oxygen consumption and the "oxygen cost" for the pumping by the heart of each milliliter of blood in the stroke volume.

A value of $PBA_{CVS} - CVS_{BA}$ below –6.3 indicates a slowing of rate of aging of the cardiovascular system; a value of $PBA_{CVS} - CVS_{BA}$ between –6.3 and 5.5 is the average rate of aging of the cardiovascular system, while a value of PBA $PBA_{CVS} - CVS_{BA}$ above 5.5 indicates an increased rate of aging of the cardiovascular system.

The simplest and most convenient definition of biological age (BA) is the definition made through the results of blood tests, in which:

$$BA = 91{,}1512 - 1.17 \bullet M + 0.5683 \bullet ESR - 0.4346 \bullet TP + 2.2088 \bullet U - 0.6613 \bullet C, R = 0.53; P < 0.001$$

where M is the monocyte count, ESR the erythrocyte sedimentation rate, TP is the total protein content of the urine, U is the concentration of urea, and C is the concentration of creatinine.

An equation for calculating proper biological age (PBA) is:

$$PBA = 53.2891 + 0.2793 \bullet HA$$

where HA is chronological age.

Piazza and coworkers have offered another system of markers that reflect aging [10], in which various markers are used to define the status of hypothalamic-adrenal regulation as an indicator of aging.

The CD4 (+)/CD25(+) subpopulation of T lymphocytes is considered an important biomarker of aging of the immune system [12], especially in persons over the age of 60 years. In elderly patients with chronic inflammatory diseases, an increase the population of monocytes with a CD14 (+)/CD16(+) phenotype may be associated with disease progression and aging, and can be considered a marker of proinflammatory and proatherosclerotic activity [13].

Allostatic load as a complex set of biological markers of aging. The term "allostatic load," originated by McEwen and Stellar in 1993 [14] denotes a dysregulated response to stress in which adrenaline, noradrenaline, and dopamine, the major hormonal mediators of stress, are secreted in excess, leading to hyperactivity of other mediators of the stress response [15]. The continuous production of these hormones over a long period can cause substantial harm to the body through. chronically increased blood pressure, a chronically high heart rate, and other effects. To study the effect of allostatic load on the mortality of 70-year-old persons over a 4.5-year period, Karlamangla and colleagues used a set of 10 biological markers [16], consisting of the waist-hip index; systolic and diastolic blood pressure; urinary cortisol, norepinephrine and epinephrine; and the serum levels of dehydroepiandrosterone; glycated hemoglobin (HbA_{1c}); high-density lipoprotein (HDL), and total cholesterol. Allostatic

Table 3–2. Description and Age-Related Changes of Biomarkers of Sympathetic Function Secreted by the Adrenal Medulla

Biomarker	*Description*	*Functions*	*Age-related Changes*	*Relationship to Disease*
Systolic blood pressure (SBP)	Maximal systolic blood pressure occurs with left ventricular systole	Indicator of cardiovascular status; is the maximum pressure on the vascular during systole that ensures the delivery of oxygen to vital organs and skeletal muscles	Increases, with possible subsequent stabilization	A high SBP reflects hypertension and coronary heart disease
Diastolic blood pressure (DBP)	Minimal blood pressure at rest	Indicator of cardiovascular status; is the minimal pressure on the vascular walls during diastole that ensures the delivery of oxygen to vital organs and skeletal muscles	May increase, with subsequent stabilization	A high DBP reflects hypertension in young persons; a lower DBP in elderly persons may indicate hypotension and the risk of dizziness/ loss of consciousness and falling
Heart rate (HR)	Number of heartbeats in a measured period (usually in minutes)	Indicator of cardiovascular status; regulates blood flow and oxygen delivery to skeletal muscles	Lowering of a maximal heart rate; heart rate stability at rest	A high heart rate indicates high blood pressure; a decreased heart rate in middle ages may indicate hypotension and the risk of falling
Adrenaline (epinephrine)	Catecholamine secreted by the adrenal medulla; stimulates the sympathetic nervous system	Sympathetic excitation in response to stress, reflected by a rapid heartbeat, glucose release, and increased blood flow to skeletal muscles	Possible stabilization or reduction in levels of adrenaline	High levels of adrenaline produce anxiety and indicate the likelihood of cardiac ischemia
Noradrenaline (norepinephrine)	Catecholamine secreted by the adrenal medulla; stimulates the sympathetic nervous system	Sympathetic excitation in response to stress, reflected by a rapid heartbeat, glucose release, and increased blood flow to skeletal muscles	Possible increase in norepinephrine levels	High levels of noradrenaline affect nthe amygdala; are involved in the "fight-or-flight" response, produce anxiety, and indicate the likelihood of cardiac ischemia; dysregulation leads to depression
Salivary alpha-amylase	Enzyme produced by the salivary gland	Breakdown of carbohydrates and starch	Reduction in scretion of the enzyme	Abnormally low levels result from poor oral hygiene

Table 3–3. Description and Age-related Changes of Biomarkers of the Hypothalamic–Pituitary–Adrenal System

Biomarker	*Description*	*Functions*	*Age-related Changes*	*Relationship to Disease*
Adrenocortico-tropic hormone (ACTH)	Hormone released by of the anterior pituitary	Stimulates the secretion of corticosteroids by the adrenal cortex	Higher levels when in a state of decline; reduced self-suppressor feedback to pituitary	High levels are a sign of Addison's disease and Izenko-Cushing's disease
Corticoliberin (corticotropin-releasing hormone (CRH)	Neuropeptide hormone released by the paraventricular nucleus of the hypothalamus	Initiates the reaction of the hypothalamic-pituitary-adrenal system to stress, stimulates the release of ACTH from the anterior of pituitary	Overactivity of corticoliberin, but reduced effect on pituitary	High levels associated with depression and cognitive decline
Cortisol	Hormone released by the adrenal cortex	Hormonal response to stress; stimulates the production of energy and gluconeogenesis; promotes carbohydrate storage; exerts an antiinflammatory effect; acts in a feedback manner to the hypothalamus to reduce its own secretion and reduces the activity of the hypothalamic-pituitary-adrenal system	Higher levels when in a state of decline; reduced self-suppressor feedback to hypothalamus	High levels indicate cardiovascular disease, depression, anxiety, and cognitive decline
Arginine-vasopressin (AVP) (also known as antidiuretic hormone (ADH)	Hormone secreted by the posterior pituitary gland	Secreted in response to stress through stimulation by ACTH; maintenance of water and salt balance	Enhanced release of ACTH; decreased sensitivity of renal collecting ducts to ACTH; loss of ACTH receptors in kidneys; neuromodulation of ACTH decreases	Reduction of concentrative function of kidneys; dysfunctional blood pressure regulation; cognitive decline
Dehydroepiand-rosterone sulfate/ dehydroepiand-rosterone	Steroid hormone secreted by the reticular zone of the adrenal cortex	Predecessor of androgens; reduces effect of cortisol to hippocampus	Decrease in hormone secretion and blood levels	Decreased levels are associated with cardiac ischemia and mental dysfunction

Note: SBP—systolic blood pressure; DBP—diastolic blood presure; HR—heart rate; ABP—arterial blood pressure

Table 3–4. Description and Age-related Changes of Biomarkers of Immune-System Function (From Alonso-Fernández and DelaFuente) [11]

Biomarker	*Description*	*Functions*	*Age-related Changes*	*Relationship to Disease*
Epithelial barrier	Skin, mucus membranes	Prevents penetration of pathogenic microorganisms into the body	Age-related decline in integrity and protective function	Increased risk of invasion by pathogens
Complement proteins	Plasma proteins activated in inflammation and which participate in defenses against pathogenic organisms	Particpate in destruction of pathogens; promote phagocytosis	Age-related decline in protein concentrations	Low levels impair desctruction of pathogens and antigens
Phagocytes Neutrophils Granulocytes Macrophages Dendrocytes	Immune cells that attack and destroy pathogens and malignantly transformed (cancer) cells in the body	Phagocytosis; initiation of the inflammatory and other immune processes	Age-related decline in ability to attack and destroy pathogens	Increased susceptibility to infection
C-reactive protein (CRP)	Protein, which amount increases during inflammation; a marker of inflammation	Increases phagocytosis, promotes binding of complement with foreign and / or damaged cells	Increase in the number of proteins with age	High concentrations reflect severity of inflammation
Natural killer (NK) cells	Cytotoxic lymphocytes that destroy invading pathogens and infected or malignantly transformed (cancer) cells	Destruction of virus-infected cells; suppression of viral reproduction; destruction of cancer cells	Reduced efficacy of action	Increased susceptibility to infection

T-lymphocytes	White blood cells produced by bone marrow and which mature in the thymus; responsible for cellular immunity	Recognition of exogenous antigens. T-helper cells release cytokines that signal other immune cells; regulatory T cells suppress immune reactions; cytotoxic T cells lyse virus-infected body cells. Memory T-cells stores information about previously existing antigens to initiate subsequent immune reactions to them (anamnestic response)	Decreased efficiency of action	Decrease in numbers and effectiveness reduce body's ability to resist infection
B-lymphocytes	White blood cells derived from bone marrow; responsible for antibody scretion and humoral immunity	Recognition of exogenous antigens; synthesize antibodies; labeling of infected cells for phagocytosis	Decreased production of antibodies	Decline in cell counts/ dysfunction weakens reactions to/efficacy of vaccines and results in ineffective response to the antigens
Proinflammatory cytokines (interleukin-6, tumor necrosis factor, etc.)	Substances that promote and participate in inflammation	Released by immune cells to initiate an inflammatory reaction; influence the differentiation of T- helper cells in the immune response	Increased number of these cytokines with age is supported by most sources	Increased level of proinflammatory cytokines may indicate various age-related diseases (osteoporosis, atherosclerosis, etc.)

load was determined twice, with an interval of 2.5 years. Patients with increased allostatic load were found to have a greater mortality than patients with decreased allostatic load. The authors stated that an increase in total score (which ranges from –1.7 to +1.4) by one point increased the risk of mortality 3.3 times over the 4.5-year study period.

The so-called Matrix Protocol of Biomarkers, used by the International Institute of Longevity in Montclair (New Jersey), represents the result of an attempt to implement a generalized approach to identifying biomarkers of aging. The protocol is designed to measure the degree of aging at four levels: the general function of the body, cellular function as reflected by skin cells, the molecular level, and DNA [17]. These four levels, and the biomarkers used in each level, are as follows:

Level 1. Biomarkers of aging at the physiological level or level of overall functioning. These include:

- The ratio of lean body mass to fat mass
- Flexibility
- Aerobic endurance
- Bone density
- Latency of the tactile reaction
- Forced expiratory volume
- Vision and hearing

Level 2. Biomarkers of aging at the cellular level, based on a biopsy of skin areas not exposed to sunlight but showing signs of skin aging. These include:

- Changes in the basement membrane
- Epidermis turnover
- the ratio of collagen
- Architecture of the sebaceous glands
- Microvascular changes
- Elastic fiber content of the skin

Level 3. Biomarkers of aging at the molecular level include key hormones, and are as follows:

- Growth hormone
- Thyroid hormone
- Coenzyme Q10
- Insulin sensitivity
- Heat-shock proteins
- blood tests on oncogenes
- Serum levels of antioxidants

Level 4. Biomarkers of aging at the chromosomal level are still being developed and tend to appear futuristic. They include the position of telomere and the rate of damage to DNA. Scientists at the International Institute of Longevity have developed a blood test that will reflect damage to DNA to reveal the exact effect of a particular anti-aging therapy on reducing the level of DNA damage in cells.

A priority area of research on biomarkers of aging is the identity of molecular markers of aging that would predict the "real biological age" of the human body and the beginning

of age-related diseases. Some known molecular biomarkers and others undergoing initial investigation and evaluation are discussed below.

3.2 MOLECULAR BIOMARKERS OF AGING

3.2.1 Telomeres and Telomerase

The lengths of telomeres in human cells, and particularly in leukocytes, has been proposed as a biomarker of aging by several authors [18, 19].

Telomeres are complexes of protein and RNA that protect the end regions of chromosomes. With each cycle of cell division, the telomeres at the ends of the chromosomes of a cell undergo shortening, which results in a "replicative aging" of cells. The correlation between short telomeres and increased mortality has been identified in many studies and is the reason for the proposal of telomere length as a biomarker of aging [20–25].

Humans, like other higher organisms, show an accumulation of DNA damage during aging [26], and it is believed that this accumulated DNA damage accelerates aging [27]. Some studies have shown that mice with defects in genes that encode reparative proteins are prone to accelerated aging [28]. One such reparative protein is the enzyme telomerase, which partly repairs the damage to telomeres and whose activity is also considered a biomarker of aging [29, 30]. Through its effect on the telomere, telomerase strictly controls the stability of the latter and is important in the survival of cancer cells and stem cells, in tissue regeneration, and ultimately in the mechanism of aging [31]. It is believed that control of the transcription of key subunits of telomerase plays a critical role in the survival of cancer cells and determines the duration of existence of nontransformed cells. Accordingly, studies of the regulation of telomerase activity hold the promise of significantly advancing knowledge about aging [32, 33].

Damage to the telomere can be determined indirectly by measuring levels of biomarkers that correlate with disruptions of telomeres. Although Jiang and Rudolph [34] have reviewed the mechanism of telomeric shortening and aging in various organs and tissues, the direct measurement of telomere length, which is a requirement for the use of these DNA-terminal structures as biomarkers, is difficult in practice, although the dynamic monitoring of the relative lengths of telomeres in blood cells may be of practical significance in this regard [35]. Moreover, damage to telomeres can be determined indirectly by measuring biomarkers that correlate of telomere damage. Rudolph and colleagues identified four proteins whose expression increased

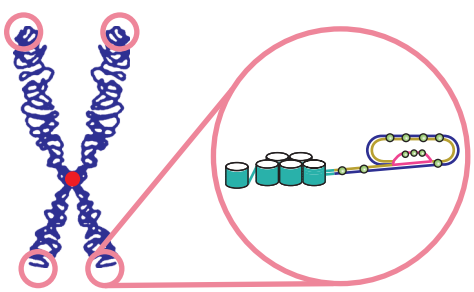

Figure 3–1. Arrangement of telomeres in a chromosome.

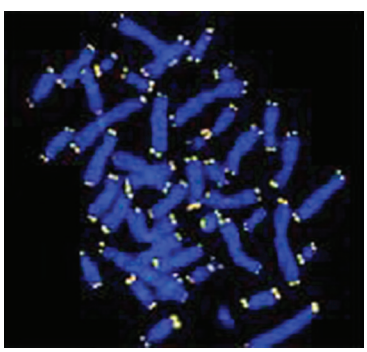

Figure 3–2. Stained chromosomes (blue) and their telomeres (white).

with telomere damage [36]. Investigation of a large and heterogeneous group of elderly subjects showed that the plasma levels of these candidate proteins as biomarkers increased with time in healthy elderly people and continued to increase with increasing age, although the exact mechanisms for these increases remains to be identified.

Potential candidates as biomarkers of aging are that sequenced fragments of the following proteins (1) Cathelicidins, a family of antimicrobial polypeptides found in the lysosomes of macrophages and polymorphonuclear leukocytes (PMNs) encoded by the cathelicidin related antimicrobial peptide (CRAMP) gene and activated by bacterial infection. Although the protein itself is not associated with age [37], CRAMP is the most commonly measured of the cathelicidins because there is a standard enzyme-linked immunosorbent assay (ELISA) for it. The plasma level of this protein correlates directly with telomere length in persons aged 25 to 78 years [45]. (2) Chitinase 3-like3 (Chi3L3), a protein belonging to the chitinase family that is associated with chondrocytes in aging and arthritis [40] and which participates in the innate immune response [38, 39]. (3) Elongation factor 1α (EF- 1α), which controls protein synthesis during the aging of human fibroblasts [41, 42]. (4) Stathmin, which monitors the stability of intracellular microtubules, cell motility, and mitosis [43]. Table 5, from an article by Jiang and coworkers [44], compares the numbers of cells with these markers in young and elderly persons.

Marker	*Young Persons (n = 6, mean age 21 ± 7.7 years)*	*Elderly Persons (n = 5, mean age, 76 ± 7.6 years)*
CAMP	0	1.8 ± 0.9%
EF-1	0	1.5 ± 0.7%
Stathmin	0	1.1 ± 0.5%

In a review published in 2005 of *in vitro* and *in vivo* studies relating to telomeres, von Zglinicki and Martin-Ruiz [46] found that telomere length satisfies several criteria of the American Federation of Aging Research [47, 48] for biomarkers of aging, in varying with age, having a high individual variability, being linked to fundamental biology, and correlating with the process of aging and the emergence of age-associated diseases.

Long-term, population-based clinical studies provide many reasons to doubt a strong correlation between telomere length and human aging [49], in that telomere length varies widely among individuals of the same age [50]. On this basis it might seem logical to conclude that telomere length and its individual differences reflect genetic and environmental factors that would explain any relationship between telomere length and biological aging. An ongoing population study of telomere length and other aging-related parameters as indicators of aging will help to resolve this issue [51]. Interestingly, however, three independent studies have found an increase in telomere length in parallel to an increase in age [52–54], although in each case this finding was made in a minority of participants. It has been suggested [55] that this may not be due to an increase in average telomere length but rather to the loss of cells with shorter telomeres. Perhaps telomere length serves as a reliable biomarker of aging only at certain stages of life or in the presence of certain diseases [56, 62]. What can be said at present is that that the length of telomeres and the rate of their shortening in the population varies greatly.

Another problem is the difficulty of determining whether telomere length is an indicator of the normal aging process or only of prodromal illness associated with aging [57]. Insufficient knowledge about the aging process continues to make it difficult to distinguish aging itself from diseases related to aging [58, 59]. Nor is sufficient information yet available about the possible effect of previous illnesses, such as viral infections, on the length of telomeres, or

whether such factors might hamper interpretation of the findings in studies of telomere length as an indicator of aging. However, results of meta-analysis indicate that telomere shortening may mark a human predisposition to cancer. In this meta-analysis, the relationship between relative telomere length and cancer risk was statistically significant [63].

There also exist both hypotheses and experimental data to the effect that telomere length is both a biomarker of aging and a determinant of life expectancy [60]. However, the direct evidence for this concept is questionable, and a systematic review by Mather et al. [61] found no credible evidence for it.

Among other candidates as biomarkers for aging are microRNAs (miRNAs), which are short, noncoding RNAs that typically act at the posttranscriptional level as negative regulators mRNA expression [64].

3.2.2 Indicators of Oxidative Damage

The concept of oxidative stress caused by free radicals or radical-generating agents in concentrations that exceed the capacity of antioxidant defenses is the basis of the free-radical theory of aging [65, 66], and is the basis for considering indicators of oxidative damage as another group of potential biomarkers of aging.

It has been established that a decreased sensitivity to oxidative stress increases longevity [67], and the inoculation of aged animals with a substance that acts as a free-radical "trap" was found to restore some of these animals' biochemical parameters to the levels found in young animals [68]. Moreover, the dependence of aging on the accumulation of free radicals has been clearly shown in model organisms [69–73]. Study of the expression of genes of *Drosophila melanogaster* under conditions of oxidative stress and aging [74, 75] found an increased expression of heat shock proteins, genes associated with the immune response, and antioxidant genes under both such conditions. It has also been shown that artificially increasing the expression of several genes involved in the antioxidant response can increase the life span of Drosophila flies [76].

Nevertheless, and despite the wide acceptance of the free radical theory as a basis for aging, strict proofs of its correctness are still lacking. With aging, oxidation by free radicals affects both nucleic acids and proteins. It is also known that damaged proteins may be involved in the pathogenesis of such age-associated diseases as Parkinson's disease and Alzheimer's disease [77]. This prevents distinguishing normal markers of aging from markers of age-associated diseases.

Carbonylated proteins are one example of using indicators of oxidative damage as biomarkers of aging. Many scientists consider the accumulation of carbonylated proteins in erythrocyte membranes as an indirect marker of human aging, and of ferric-reducing antioxidant potential (FRAP) in the blood plasma as an indicator of antioxidant defense [78–80]. The degree of increase in carbonyl groups in proteins in the membranes of red blood cells as an indicator of aging is shown in Figure 3–3, published by Jhaetal [81].

3.2.3 Antioxidant Enzymes as Biomarkers of Aging

In addition to the participation of enzymes with reduction/oxidation potential, such as glutathione (GSH) reductase and thioredoxin reeducates in cellular antioxidant defenses, there is evidence that these enzymes share in several functions necessary to cell growth and viability, including participation in the regulation of transcription factors and of the cell cycle regulation, as well as in the inhibition of apoptosis [82]. A 5-year follow-up study of swallows showed that a high level of antioxidant protection was positively correlated with these birds' survival

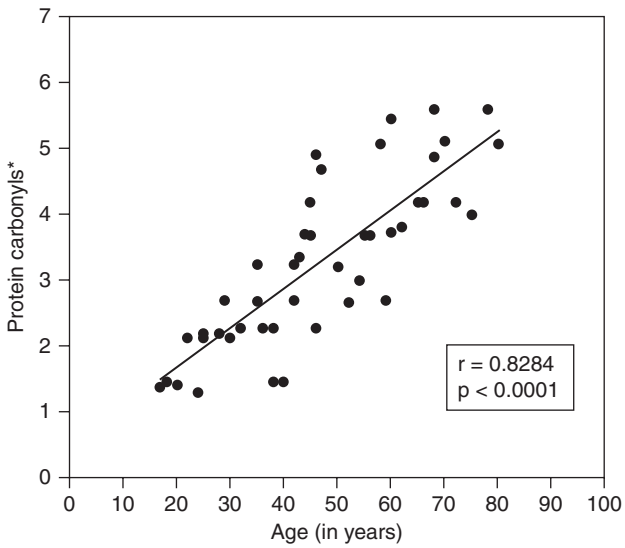

Figure 3–3. Carbonyl groups of proteins in the erythrocyte membrane as a function of age. *Carbonyl-group protein is measured in nanomoles per milligram of protein.

and longevity [83]. The activity level of oxidative and reductive enzymes acting on glutathione, thioredoxin, and various other substrates may not only reflect the level of an organism's antioxidant defenses, and are probably biomarkers of normal aging as well as biomarkers of age-associated diseases [84–86].

3.2.4 Consideration of the Level of Mitochondrial Microheteroplasmies as Potential Biomarkers of Aging

Microheteroplasmy is the presence of hundreds of independent mutations of mitochondrial DNA in a single organism, with each mutation occurring in 1% to 2% of all of the organism's mitochondrial genomes [87, 88]. Despite this low incidence of single mutations, most of the mitochondrial genomes of all adult members of the organism have mutations. This "burden" of mutations includes hereditary mutations as well as mutations occurring de novo during embryonic development and somatic mutations. It has been suggested that microheteroplasmy can explain the pathologic mechanisms of age-related diseases such as diabetes, cardiovascular disease, Parkinson's disease, Alzheimer's disease, and cancer.

Her discovery of mobile genetic elements (MGE) led to the American geneticist Barbara McClintock's being awarded the 1983 Nobel Prize in Physiology. MGE have been proposed as components of genetic instability, and on this basis as factors in the aging of cells of many living species [89, 90], with effects including deregulation of gene expression and age-related disruption of cell physiology, arrest of cell growth, and eventual cell death or blast transformation.

According to one hypothesis, aging and cell death are at least in part due to the process of transposition, in which one copy of a sequence of DNA in the nucleus of a cell in remains in place while the second copy of this DNA, known as a transposon, moves to another location in the genome. Repetition of this transposition of DNA sequences within a strand of DNA follows an exponential course in which the number of transposons ultimately inactivates essential genes and leads to the death of the affected cell line or of the entire organism [91].

The correlation between aging and transposon activity has been investigated in a number of biological systems [92–94], and it can be expected that study of the mechanisms regulating the

generation and transposition of MGE will reveal therapeutic targets for combating premature aging and many pathological processes associated with genomic instability.

3.2.5 Levels of Activity of NADH and FAD as Biomarkers of Aging

The coenzymes nicotinamide adenine dinucleotide (NAD) and flavine adenine dinucleotide (FAD) play key roles in the transport of energy for cellular metabolism. In their reduced forms of NADH and FADH, respectively, these coenzymes transport energy along the mitochondrial electron transport chain, ultimately giving up their energy, through the breakage of their hydrogen bonds, to energize various cellular metabolic reactions. In this process of giving up their hydrogen atoms, NADH and FADH return to their oxidized forms of NAD and FAD, in which they can then once again take on hydrogen atoms and re-enter the energy-transport chain.

Because NADH and FADH are critical components of the mitochondrial transport of energy, their intracellular concentrations can be considered potential biomarkers of mitochondrial activity and, through the cellular reactions that depend on mitochondrial energy transport, of cellular senescence and the aging of an organism [95]. The accurate measurement of these coenzymes is made through the techniques of fluorescence microscopy and polarizing microscopy [96–98].

3.2.6 Expression of the Gene for Heat Shock Protein-16.2 As a Potential Biomarker of Aging

In accord with the hypothesis that markers of stress may be biomarkers of aging in mutant nematodes and other model organisms [99, 100], Johnson and associates proposed that interventions that increase the response to stress might increase longevity [101]. In work related to this question, Rea and colleagues [102] found that variations in the level of a green fluorescent protein (GFP) produced by the gene for this protein when coupled to the gene that encodes heat shock protein-16.2 (HSP-16.2), predicted as much as a fourfold difference in the longevity of the nematode worm *Caenorhabditis elegans*. Although the gene for HSP-16.2 is not itself responsible for such differences in the survival of *C. elegans* under experimental conditions, stress causing variations in the expression of this gene is likely to govern the longevity of this worm.

3.2.7 Apolipoprotein Content as a Biomarker of Aging

The substances known as apolipoproteins, synthesized in the liver and intestine, are involved in the transport of cholesterol and triglycerides through the blood to and from various tissues and organs of the body. Apolipoproteins are significantly more accurate predictors of death from coronary heart disease in than are routine lipid measurements. In particular, apolipoprotein A-1 transports high-density lipoproteins (HDL), of which increased levels in the blood have been found to correlate strongly with a reduced risk of death from coronary heart disease (CHD), and apolipoprotein B transports low-density lipoproteins (LDL), increased levels of which have been found to correlate strongly with an increase in such risk. In a study involving 30,819 men, women, and children ranging in age from 20 to 89 years, conducted as part of the Third National Health and Nutrition Examination Survey (NHANES) [103, 104], Sierra-Johnson and coworkers found that the blood level of apolipoprotein B had a greater prognostic value for death from CHD than did the level of apolipoprotein A-1 or

any other apolipoprotein (Sierra-Johnson J, Fisher RM, Romero-Corral A, et al. Concentration of apolipoprotein B is comparable with the apolipoprotein B/apolipoprotein A-1 ratio and better than routine clinical lipid measurements in predicting coronary heart disease mortality: Findings from a multiethnic U.S. population. *Eur Heart J* 2008;30(6):710–717.) It is believed that the determination of apolipoproteins can be used in study of the aging process as well as the diagnosis of atherosclerosis and related diseases.

3.2.8 Interleukin-6 Level as a Biomarker of Aging

Interleukin-6 (IL-6) is a multifunctional cytokine that plays an important role in the acute phase of inflammation. In the normal state in which inflammation is absent, IL-6 is not expressed and not detected in the blood. However, it begins to be expressed with the beginning of aging, in association with the age-dependent loss of its normally regulated expression [105, 106]. A study of 473 elderly men [107] found an inverse relationship between levels of testosterone and of the IL-6 receptor, the which reflects the activity of IL-6, but no relationship with other markers of inflammation. The study concluded that increased activity of IL-6 in the absence of changes in other markers of inflammation suggests a close relationship between a proinflamatory state and a decline in testosterone levels, both of which are associated with age-related changes in men. Klein and colleagues found a link between the increased expression of IL-6 and age-related cataracts [109], and a link has been suggested between increased levels of IL-6 and an increased risk of mortality in elderly persons [110]. Another study suggested a relationship between hormone levels and age-related frailty in persons over the age of 80 years, and noted that because such frailty involves multiple systems, there is a need for measures of endocrine function that can distinguish its specific relationship to both endocrine-related disease and aging [108].

3.2.9 Growth Hormone During Aging as a Marker of the Aging Process

Hormones bind to receptors through which, via the mediation of various signaling intracellular pathways, they exert specific effects on the expression of various genes. Consequently, age-related changes in such signaling pathways, beginning with changes in the concentration of a particular hormone in the blood and ending with the effect of the peptide or protein on the function of cells and tissues, reflect phenotypic features of aging. The most widely discussed candidate hormone as a biomarker of aging is growth hormone (GH), on the basis of the well known fact that the level of this hormone decreases with age. Secretion of GH by the pituitary gland, where the hormone is synthesized, declines by about 14% per decade beginning at the age of 20 to 25 years, and is halved by the age of 60 years. At the same time, receptors for GH become less responsive to the hormone. It is thought that this reduction in the level of GH is responsible for age-dependent accumulation of adipose tissue and reduced muscle mass, as well as a decrease of the mineral content in bones [111–113]. Accordingly, it can be concluded that reduced levels of GH are associated with and contribute to the effects of aging. Alternatively, it is possible that the decline in levels of GH with aging is caused by other processes caused by aging. For example, it has been found that mice with impaired pituitary function live longer than control mice, and that overproduction of GH reduces the longevity of mice [114, 115]. Mice selected for slow growth, reflected by a lower body weight resulting from decreased secretion of GH during the first 2 months of life, exhibit reduced longevity [116, 117]. These studies provide a basis for further research on the level of GH as a marker of aging.

3.2.10 Glycation of Proteins as a Potential Biomarker of Aging and Age-associated Diseases

Advanced glycation endproducts (AGE), the final products of the nonenzymatic glycosylation of proteins known as glycation, where carboxy groups of carbohydrates form covalent organic bonds with the amino groups of proteins. The appearance of AGE in cells has been particularly linked with certain age-associated diseases, particularly type II or non-insuling-dependent diabetes mellitus (NIDDM). The accumulation of AGE has been found in different tissues of elderly patients with diabetes [118, 119], and AGE have been found to accumulate in healthy but aging people [120].

3.2.11 Gene Expression as a Biomarker of Aging

The analysis of human gene expression through microarrays of DNA, or "gene chips," has provided considerable data about genes whose expression changes with age [121, 122]. For example, Golden and colleagues [123], in work directed at identifying markers of aging in the nematode *C. elegans*, found strong correlations between changes in the expression of various genes and chronological and biological age. They developed a method that can define the biological age of wild *C. elegans* worms with 70% accuracy. The expression of p16INK4a, a protein present in human T-lymphocytes and which correlates with biological age, increases exponentially with age [124]. A further finding was that increased levels of this protein were associated with both smoking and physical inactivity [124]. This indicates once again that the level of expression of some genes makes them clear prospects as biomarkers of aging.

3.3 BIOMARKERS ATTRIBUTABLE TO SPECIFIC DISEASES OF AGING

3.3.1 β-SiteAPP-cleaving Enzyme (BACE)

The enzyme known as β-siteAPP-cleaving enzyme (BACE), expressed throughout the body and especially in the brain and pancreas, belongs to a group of membrane-bound aspartyl proteases involved in the production of the Aβ amyloid peptide that is deposited in the brain in Alzheimer's disease [125]. Other proposed markers of Alzheimer's and other neurodegenerative processes include the levels of total homocysteine (tHcy), insulin-like growth factor 1 (IGF-1), interleukin 1β (IL-1β), and tumor necrosis factor-α (TNF-α) [126].

3.3.2 N-acetyl-L-Leucine

The acetylated amino acid N-acetyl-L-leucine in blood plasma may be a marker of type II diabetes [127]. In a study at Johns Hopkins University in Baltimore, the measure known as prostate specific antigen velocity (PSAV), which reflects the rate of scretion of prostate specific antigen (PSA) in nanograms per milliliter of serum per year, was found to be associated with an increased risk of death from prostate cancer more than 10 years before its diagnosis, and may serve as a biomarker of aging, prostate cancer, and death [128].

Thus, although no universally recognized biomarker of aging has yet been found, it seems reasonable to conclude that the best biomarkers of aging are diseases associated with aging,

and that combinations of different physiologic and pathophysiologic variables are likely to represent the most reliable markers of aging. There seem to be no reasons to disagree with the view of LeCouteur and colleagues [129] that the aging process is becoming an increasingly attractive target for the development of new drugs, which will increase the demand for identifying biomarkers of aging as surrogate indicators for the effects of new medications on the aging process.

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Chapter IV

Geroprotectors

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In English scientific literature, interventions that may delay the development of age-related changes are commonly called "anti-aging medicine." English literature, pharmacological substances that can theoretically extend life are widely called "anti-aging drugs," which in the Russian literature corresponds to the term "geroprotectors" (literal translation, "protecting against old age"), a general name for a group of substances that have shown the ability to prolong the life of animals.

It is still unwise to assume that a particular, known geroprotector will slow, stop, or reverse the human aging process. Compounding doubts about the efficacy of intervention in the aging process is the lack of a generally accepted definition of the aging and of standards for biomarkers that can measure the rate of the aging process. Nevertheless, there appear good reasons to continue searching for them to improve human health and well-being.

The possibility of extending life under experimental conditions has been demonstrated for many geroprotectors, including antioxidants, chelating agents, latirogens (substances that prevent the formation of crosslinks, particularly in the collagen molecules of connective tissue), adaptogens, neurotropic drugs, monoamine oxidase (MAO) inhibitors, glucocorticoids, dehydroepiandrosterone (DHEA), sex hormones, growth hormone (GH), melatonin, pineal gland preparations, inhibitors of protein synthesis, antidiabetic agents, thymic hormones, immunomodulators, and enterosorbents (such as activated charcoal), as well as mimetics of superoxide dismutase (SOD) and catalase [1–3]. Many natural supplements, and synthetic drugs, and especially certain antioxidants, vitamins, and hormones, have in recent years been introduced to the mass market, despite the lack of solid scientific evidence of their effectiveness [4]. According to many authors, there is no chemical geroprotector with a positive effect against aging that has been indisputably proved, even though such agents can clearly exist in principle [5]. It would seem that among the key reasons for which a "true" geroprotector has not yet been developed is the lack of a method for clearly identifying the specific effect of such a substance under experimental conditions. Historically, the only criterion for judging the effectiveness of any geroprotectors has been an increase in the longevity of experimental animals. However, such an increase is unlikely to be an effective measure of the true geroprotective effect of a particular substance.

A prolongation of life in experimental animals has repeatedly been found with DDT, radioactive dust, and some other substances considered to be toxic [6], without any consideration of these substances as "geroprotective." The mechanism of such effects is hormesis, a positive effect of small doses of certain substances that in large doses have a negative effect on the body [7]. Hormesis has repeatedly been demonstrated in experimental animals with the addition to feedstuffs of such substances as herbicides, pesticides, insecticides, hydrocarbons, ethanol, and solvents [8]. In recent years, work in gerontology has actively addressed the potential for hormetic effects [9, 10], and some experimental and epidemiological studies have shown that hormesis could be an effective tool for counteracting various age-related pathologies including diabetes, cancer, and cardiovascular and neurodegenerative diseases. It has also been shown that molecules with extracellular signaling effects, such as oxygen, carbon monoxide, nitrogen oxide, the neurotransmitter glutamate, calcium ion, and tumor necrosis factor play important roles in hormesis [11].

In recent years, cellular and molecular mechanisms of hormesis became subjects of active investigation. Mechanisms found to play an important role in its manifestations include signaling pathways for growth factors, the synthesis of heat shock proteins and sirtuins, and the induction of antioxidant and reparative systems, activation of membrane receptors, stimulation of the immune system, compensatory cell proliferation, and some other processes [12, 13]. The experimental increases in longevity described earlier in relation to hormetic effects of chemical substances such as antibiotics, herbicides, pesticides, heavy metals, and hydrocarbons were also found with various "soft" stresses (eg, radiation, cold and heat shock, and hypergravity) [14, 15].

It is generally assumed that the ability of geroprotectors to prolong life is related to their specific effect on mechanisms that determine the rate of aging of cells or organisms. An alternative explanation for these effects may be the induction of adaptive hormetic responses in organisms undergoing stress. In the modern gerontological literature, it is generally accepted that the beneficial effects of geroprotectors are explained by their specific effects on mechanisms that play a role in determining the rate of aging. Thus, for example, and in accordance with the free radical theory of aging [16], free radicals produced during metabolism damage DNA, proteins, membranes, and other cell structures, leading to age-related decreases in cellular and bodily functionality, but antioxidants may neutralize free radicals and slow such processes. Because the majority of antioxidants are multifunctional, with vitamin C, for example, having the potential to act as an antioxidant, a chelating agent, a reducing agent, and an oxygen scavenger [17], the effects of geroprotectors are unlikely to be consequences of their actions on a single specific geroprotective mechanism.

As of the beginning of 2011, more than 30 substances with geroprotective properties had been described. Among them are:

- Resveratrol and other polyphenols of plant origin
- Rapamycin
- Antioxidants (vitamins A, C, and E; carotenoids; lipoic acid; coenzyme Q; the trace element selenium; and others)
- Succinic acid (amber acid)
- Inhibitors of protein biosynthesis (olivomycin, actinomycin)
- Hormones (GH, thyroid hormones, adrenal hormones, sex hormones, melatonin)
- Peptide bioregulators (timalin, epithalamine)
- Biguanides (metformin, fenformin)
- Adaptogens (ginseng and eleutherococcus)

In contrast to geriatric remedies intended for the treatment of diseases of aging or for improvement in the quality of life with aging, geroprotectors can be used in young and middle-aged persons. However, the safety of their long-term use requires further study.

A significant number of geroprotectors have been proposed on the basis of the various different theories and molecular mechanisms proposed for aging, but as noted earlier, none has been found to correct or modify any specific mechanism of aging. Published data on geroprotectors are fragmentary, contradictory, and often unreliable both in terms of the adequacy of investigations of their effects and the interpretation of experimental findings, and few substances have shown even promising geroprotective properties.

4.1 THE EFFECT OF REDUCING CALORIC INTAKE: CALORIC RESTRICTION AND ITS MIMETICS

It has been suggested that reducing caloric intake by 30% to 50% below an ad libitum level of intake may delay aging and aging-related diseases, and provides increased resistance to stress and a delay in functional decline [18]. It las long been known that the reduction of food intake by various experimental animals, from invertebrates to mammals, can prolong their lives. Such caloric restriction (CR) is still the most reliable and reproducible method of increasing the lifespan of many animals, including mammals and even nonanthropoid primates under experimental conditions, and this would seem logically to extend to humans [19–22]. The effects of CR on mammals include the prevention of major age-related diseases, including tumors, diabetes, cardiovascular disease, and neurodegeneration. This is accompanied by the suppression of chronic inflammatory reactions and degenerative processes in tissues [23]. Short-term studies in humans have shown a decreased risk of cardiovascular disease and improved insulin sensitivity with reduced caloric intake [24, 25].

Although the paradigm of CR has been known for over 60 years, precise biological mechanisms for this effect and its general applicability to humans remain disputable. In a controlled study of CR in monkeys, Roth and colleagues [26] obtained results strongly suggesting that the "anti-aging" or "antidisease" effects of such restricton observed in rodents are reproduced in primates. They include reduction of the plasma insulin level and increased sensitivity of insulin receptors; decreases in body temperature, cholesterol, triglycerides, alpha-lipoproteins, and blood pressure; and increases in serum HDL. Taken together, these findings give reason to suppose that CR in primates can reduce their risk of diabetes, cardiovascular disease, and other age-related diseases, and that these findings also extend to humans, although it is unlikely that most people would be willing to support a 30% reduction in food intake even if this would mean an increase in healthy years of life. There are assumptions about the mechanisms explaining the possibility of extending the life situation saloric restriction, for example, Shin-ichiro Imai [27], suggests a connection with the effect of CR sirtuins.

Although a number of items in Figure 4–1 have not been proven relevant at the human level, the results of experiments with other animals, and rodents in particular, allow the extrapolation of its fundamental components to a relationship of CR to sirtuins and longevity in humans.

A major role in the regulation of the processes linking CR to sirtuins and thence to longevity is attributed to the *SIR2* gene, different versions of which are found in all organisms from yeast to humans. It has been found that nutrient deficiency in yeast triggers the mechanism illustrated in Figure 4–1, which increases the enzymatic activity of Sir2, the protein encoded by the *SIR2* gene [28, 29]. The next step in this *SIR2*-regulated pathway is activation of the *PNC1* gene, which encodes nicotinamidase, an enzyme that deaminates nicotinamide (NAD), a substance essential to mechanisms governing the cellular generation of energy through its repeated cyclic reduction to NADH and subsequent reoxidation to NAD. In addition to this, NAD participates in mechanisms regulating stress resistance and longevity, and normally also downregulates the activity of the *SIR2* gene through feedback inhibition of its expression. Because of this, any change in the intracellular ratio of NAD to NADH, such as that induced

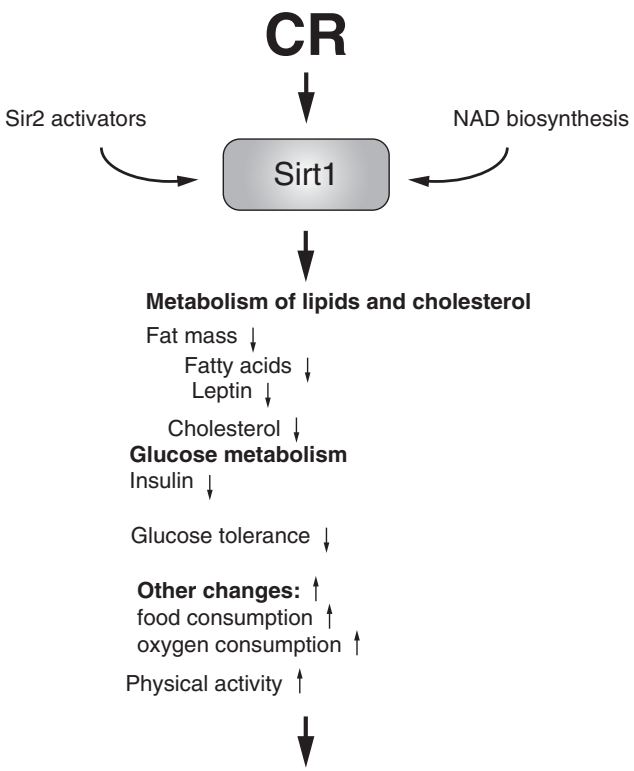

Figure 4–1. Extending lifespan.

by CR, which increases the activity of the Sir2 protein (Figure 4–2). In mammals the equivalent of the yeast *SIR2* gene is the *SIRT1* gene, which operates through a more complex mechanism than that described above for lower animals.

4.2 SIRTUINS

Sirtuins are deacetylase and ADP-ribosyltransferase enzymes [30]. Their name was derived from that of *Saccharomyces cerevisiae*, in which they are encoded by the *SIR2* gene and which was the first living organism in which they were identified. As noted earlier, the main effect of

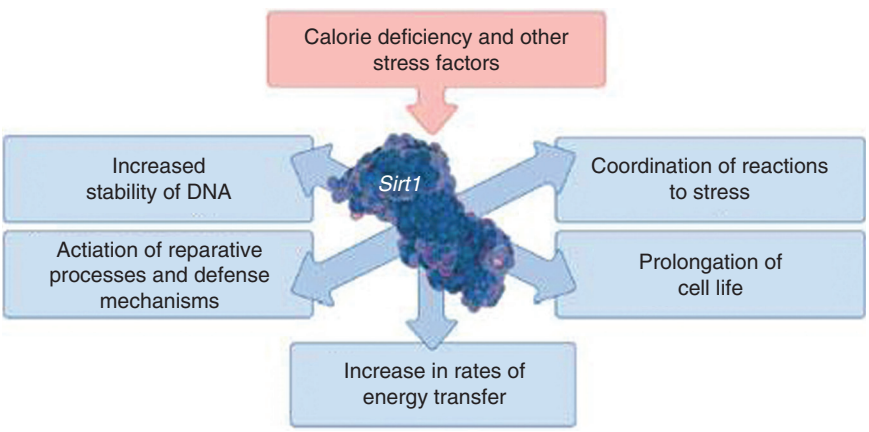

Figure 4–2.

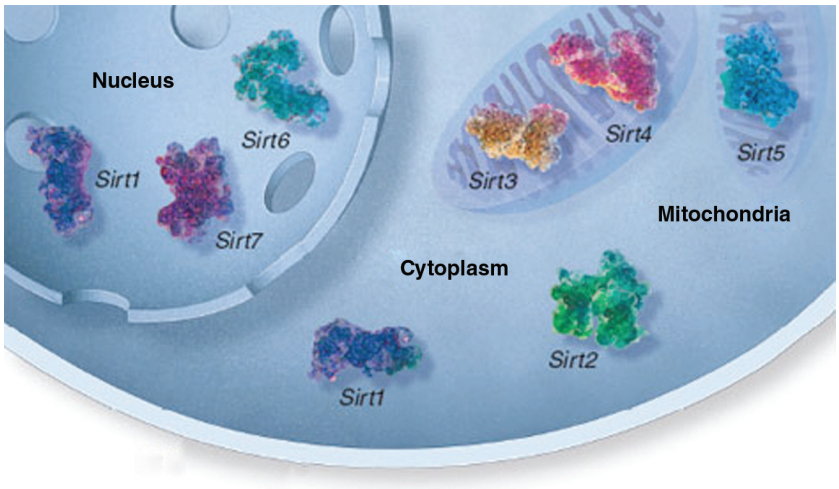

Figure 4–3. Locations of sirtuins in human cells.

CR on longevity is realized through sirtuins, with a connection between increased expression of these enzymes in yeasts and worms and increased longevity in these organisms [31–34].

The specific effects of the seven known mammalian sirtuins, Sirt1 through Sirt7, have not yet been fully ascertained, and their effects on longevity remain unknown, although CR increases the expression of Sirt1 through Sirt3 and of Sirt6 in different tissues [35, 36]. The hypothesized locations of sirtuins in human cells include the cytoplasm, cell nucleus, and mitochondria, and are represented schematically in Figure 4–3.

Sirt1 is one of the most intensively studied proteins of the sirtuin family, and is found in the nucleus and cytoplasm. Many of its targets are transcription factors that activate genes and thus regulate their activity, giving it a role in a wide range of important intracellular processes, and it also deacetylates other proteins, thereby changing their behavior. Recent researches on the role of other proteins in the sirtuin family [38] has revealed that Sirt2 modifies tubulin, the protein constituting the cellular microtubules that maintain cells' structures and play important

Table 4–1. Cellular Location and Function of Mammalian Sirtuins (from reference [37])

Sirtuin	*Location*	*Enzymatic Activity*	*Function*
SIRT1	Nucleus	NAD-dependent deacetylase	Metabolism/aging/cancer/neural differentiation/rRNA synthesis
SIRT2	Cytoplasm	NAD-dependent deacetylase	Cell cycle/adipogenesis/ neurodegeneration
SIRT3	Mitochondria	NAD-dependent deacetylase	Mitochondrial deacetylation
SIRT4	Mitochondria	ADP-ribosyltransferase	Mitochondrial deacetylation/insulin metabolism
SIRT5	Mitochondria	NAD-dependent deacetylase	Mitochondrial deacetylation
SIRT6	Nucleus	NAD-dependent deacetylase	Maintains telomeric stability/genomic stability
SIRT7	Nucleus	ADP-ribosyltransferase	Stress resistance (heart)/RNA pol1-mediated transcription

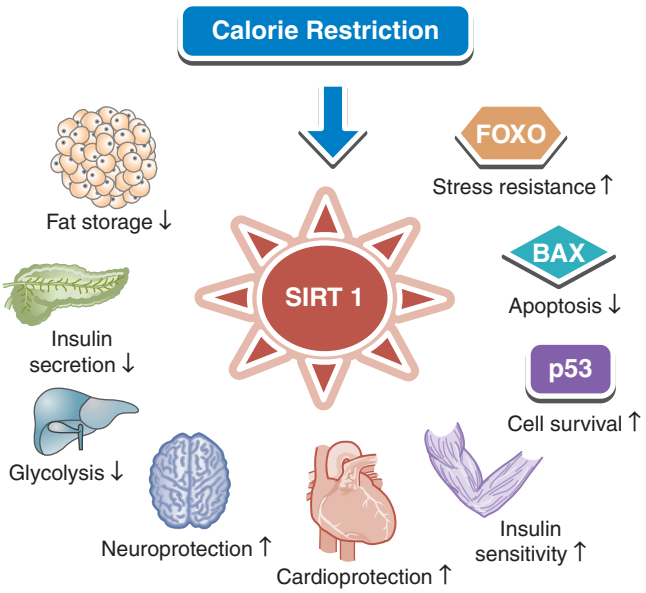

Figure 4–4. A possible interaction of CR with Sirt1, and its participation in regulating metabolc processes connected with aging. (From Eun-Joo Kim and Soo-Jong Um [39])

roles in intracellular transport and cell division. Sirt3 influences energy production in mitochondria and appears to participate in the regulation of body temperature. The functions of Sirt4 and Sirt5 have not yet been determined, but mutations in the gene that encodes Sirt6 result in premature aging. Fox01, Fox03 и Fox04: transcription factors influencing on activity of protective system of a cell and glucose metabolism. Histones H3, H4 and H1: participate in packing of the DNA in chromosomes. Ku70: a transcription factor promoting the DNA repair and cell division. MyoD: a transcription factor promoting muscles formation and elimination of tissue damages. NcoR: regulates activity of many genes including those which influence on fats metabolism, inflammatory processes and functioning of other regulatory proteins such as PGC-1. NF-κB: a transcription factor participating in regulation of inflammatory response, survival rate of cells and their growth. P300: a regulatory protein participating in acetylization of histones. P53: a transcription factor initiating apoptosis of damaged cells. PGC-1: regulates cell respiration process and apparently plays a key role in development of muscles.

A direct interaction between CR and human sirtuins, and particularly SIRT1, is shown in Figure 4–4.

It has been hypothesized that CR or cell stress increases the activity of SIRT1, which regulates metabolic changes connected with aging, including an increased mobilization of fat in white fat tissue [40], a decrease in insulin secretion and increase in insulin sensitivity in the liver and muscles [41], neuroprotection against axonal degeneration and Alzheimer's disease [42, 43], cardioprotection and the protection of cardiomyocytes from ischemia-induced apoptosis [44], and the potentiation of stress resistance and preventionj of stress-induced apoptosis [45]. All of these findings support the concept that SIRT1 regulates important physiological processes involved in CR-mediated mammalian longevity.

Because it is unlikely that most people will be able to adopt a lifestyle based on CR in view of its effect on established habits and the risk of depression of immune and reproductive function, there has been great interest in developing drugs that replicate some effects of CR without the need to dramatically limit caloric intake [45].

The strategies proposed for this include the inhibition of glycolysis (2-deoxyglucose) [46], potentiation of the effect of insulin (eg, with metformin) [47], and use of small molecules that activate SIRT1 (eg, resveratrol) [48].

Theoretically, the opportunity for developing CR-mimetic drugs has a sufficient foundation in the findings of research studies. The results of research on the the sirtuins indicate that they can be considered key targets in developing life-prolonging CR-mimetic agents, although much remains to explore in this endeavor.

Howitz and coworkers [49] conducted a screening of SIRT1 activators on the basis of studies indicating that sirtuins mediate a positive effect of CR on longevity, and determined that resveratrol is such an activator. Resveratrol is a polyphenol found in red wine, which was historically supposed to promote health and prevent cancer.

A term now frequently used to designated substances that work by activating SIR genes is "sirtuin-activating compounds" (STACs). Such substances include resveratrol and fisetin, as well as a number of other naturally occurring plant polyphenols, and number of other small molecules [50].

4.3 RESVERATROL AND POLYPHENOLS OF PLANT ORIGIN

Resveratrol (3,5,4-trihydrostilbene), which is today the most known geroprotective substance in the world, was discovered in 2003. It is a natural phytoalexin discharged by some plants as a defense against pathogens such as bacteria or fungi, and is found in more than 70 different species of plants. Resveratrol occurs in two isomeric forms, *cis*- and *trans*-resveratrol, of which the *trans*- form is used in research because of its greater activity.

A high concentration of resveratrol is found in grape skins, grape seeds, and grape stems, and in root of *Polygonum cuspidatum*, or fleeceflower knotweed, a traditional medicinal plant in Chinese and Japanese folk medicine. Resveratrol is present in high concentrations in red wine, with an average concentration of 5 mg/L [51]. However, it is more profitable to extract resveratrol from less costly sources, such as *P. cuspidatum*. Resveratrol has also been made synthetically.

Resveratrol is one of the most interesting geroprotective substances to have been investigated. A recent review by Wang and associates [52] called its most interesting properties, apart from an antiaging effect, its anticarcinogenic activity, antimicrobial and antiviral properties, ability to moderate dislipidemia and adiposis, potential for decreasing hyperglycemia and hyperinsulinemia, and possible protection of endothelial function.

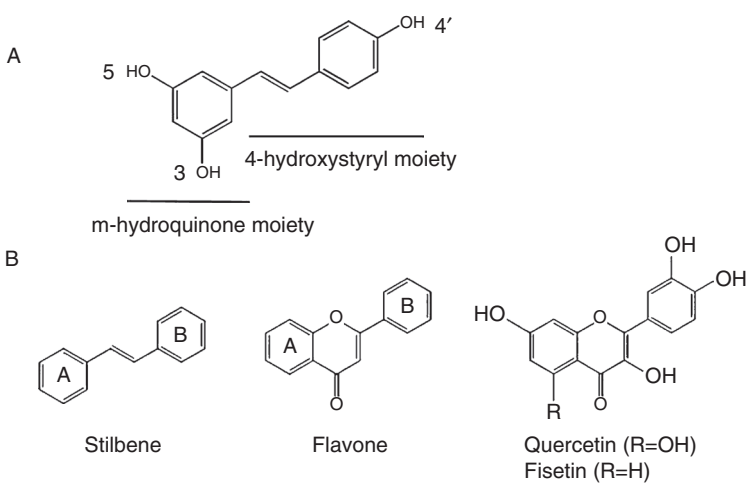

Figure 4–5. Chemical structures of resveratrol (3,4,5-trihydroxy-*trans*-stilbene) (A), and of the related substances stilbene, flavone, and the flavone derivates quercetin and fisetin (B).

In general, more than 2000 studies of the efficacy of resveratrol have been conducted at research centers around the world. To confirm the reliability of the results of studies of potentially geroprotective substances in mice, investigators at the U.S. National Institute on Aging have developed a program for the simultaneous study of such substances in three laboratories. The first group of substances to be studied in this program includes green tea extract and resveratrol.

The pharmacokinetics of resveratrol is marked by a number of peculiarities. Resveratrol has a low water solubility. As a pharmacological substance it has a low bioavailability and poor stability. It oxidizes under conditions of light and heat, which reduces its desired properties. It also has poor bioavailability owing to an acute first-pass effect in its metabolism in the gastrointestinal tract and liver and its rapid elimination from the body in the urine and feces. The pharmacokinetic curves for resveratrol and its metabolites are shown in Figure 4–7, published by Brown and associates [53].

As seen in Figure 4–7, the metabolism of resveratrol leads to glucuronates and sulfates of the parent compound within approximately 8 to 18 minutes after the entry of resveratrol into the blood, in which its concentration barely reaches the micromole-per-liter level in serum. In contrast, its metabolites circulate in the blood serum for up to 9 hours. Its low concentration in the systemic circulation significantly limits the therapeutic potential of resveratrol, making clear a need to modify its pharmacokinetics, initially through the use of transport systems that will enhance its serum concentrations [54]. It has been determined that resveratrol itself reaches its highest concentration in tissues of the large intestine, which may serve as a foundation for its potential use in preventing colon cancer [55].

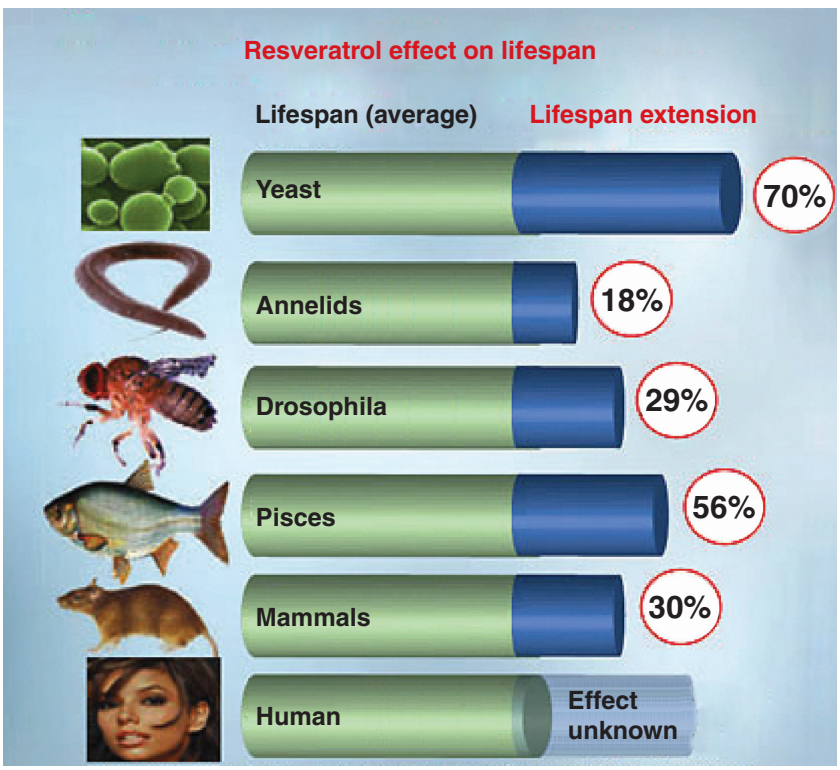

Figure 4–6. How resveratrol prolongs life. Average longevity. Extended longevity. Yeasts Annelid worms. Drosophila. Fishes. Mammals. Human being. The extent of prolongation of longevity in humans is unknown.

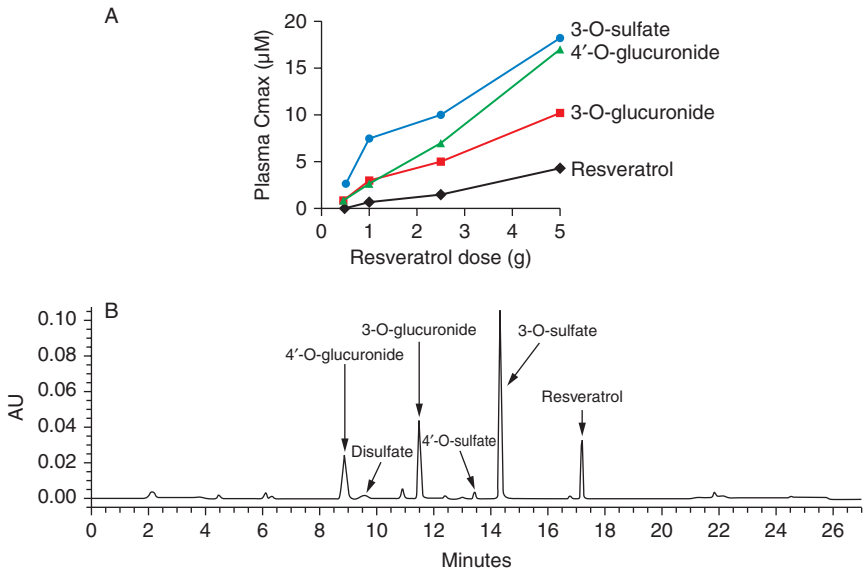

Figure 4–7. Pharmacokinetics of resveratrol in healthy volunteers at repeated dosing (A). The dosage of resveratrol was 0.5, 1.0, 2.5, or 5.0 g/day for 29 days, and its disposition was based on measurements of the urinary concentrations of resveratrol and its metabolites from days 21 to 28 of dosing. Resveratrol metabolites (B). Typical profile of resveratrol and its metabolites in the blood plasma of healthy volunteers who took 2.5 g/day of resveratrol.

The less-than-optimal pharmacokinetic properties of resveratrol have led to its modification into trans-resveratrol and the resveratrol derivatives shown in Figure 4–9, which have better pharmacokinetics and bioavailability than resveratrol itself [56].

As seen in Figure 4–9, the levels of the resveratrol derivatives SRT1720, SRT1460, and SRT2183 in blood are significantly greater than those of the parent compound.

As already noted, preclinical studies determined that resveratrol prolongs the longevity of some species of insects and other animals. The longevity of the yeast *S. cerevisiae* was prolonged by 70% through cultivation in a medium containing 10 mM resveratrol [57], and the longevities of the nematode *C. elegans* and the fruit fly *Drosophila melanogaster* were prolonged by 20% and 29%, respectively, by treatment with 100 mM resveratrol [58, 59], although another study failed to find a statistically significant effect of resveratrol on the longevities of worms and flies [60]. A study in which the fish *Nothobranchius furzeri* were fed resveratrol at 120 mg/g fishes for a typical period of 2 months found that they experienced a 50% increase in longevity [61].

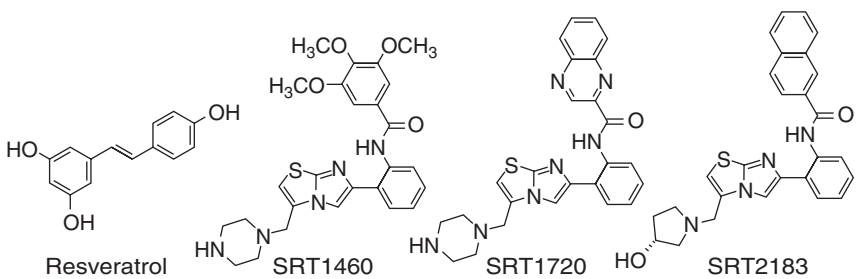

Figure 4–8. Resveratrol derivatives synthesized by Sirtris Pharmaceuticals Inc.

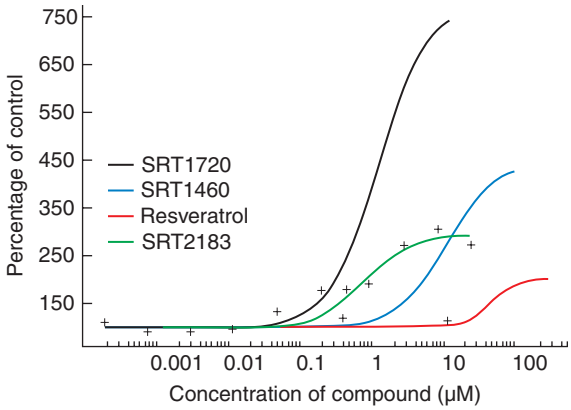

Figure 4–9. Comparative levels of the resveratrol derivatives SRT1720, SRT1460, and SRT2183 in blood.

Mice fed a high-calorie diet and resveratrol in a dose of 22.4 mg/kg lived significantly longer than controls, with longevities of up to 114 weeks, owing to enhanced glucose tolerance [62]. The effect of resveratrol on longevity in mice was considered to be at least in part connected to an increase in the activity of SIRT1 and AMPK in these animals [63].

In other studies, mice given resveratrol had decreases in such signs of aging as albuminuria, inflammation, vascular endothelial apoptosis, decreased aortic elasticity, declines in motor skills, and cataract development. In these studies, resveratrol was found to prevent genomic instability and to hamper age-related changes in gene transcription by upregulating SIRT1 in the liver and in beta cells of the pancreas, and upregulating SIR T2 [75, 76], with this same effect found for SIRT3, a key mitochondrial deacylase [77]. It is also thought that resveratrol increases the sensitivity of insulin receptors in mice by stimulating the expression of Sirt1-PGC-1α, with deacetylated PGC-1α subsequently activating oxidative phosphorylation by activating nuclear respiratory factor-1 (NRF-1).

In cultures of human MRC5 fibroblasts, which are widely used in aging experiments, resveratrol in a concentration of 5 μM and with a long incubation period showed a protective effect against oxidative damage to DNA and decreased the age-related enlargement of cell nuclei, as well as reducing the generation of the acetylated forms of histones H3 and H4, whose production has been found to be related to aging, and of p53 protein [64]. In another study of cultured human fibroblasts, resveratrol in concentrations of 10 μm to 25 μm delayed age-related morphological changes, whereas its principal metabolite, dihydroriboflavin had no effects at concentrations of up to 100 μm [65]. Contrasting with such findings, and again emphasizing that the mechanism by which resveratrol extends longevity, are findings that question the possibility of extending longevity in *D. melanogaster* and *C. elegans* through CR, the activation of Sir2, or the use of resveratrol [66–70].

It is supposed that polyphenols such as resveratrol inhibit growth by activating cell-specific genes that encode mitogens such as P13k and AKT. These work through pathways influenced by the target of rapamycin (*TOR*) or *FRAP1* gene, which in turn encodes a protein that regulates cell growth, proliferation, survival, and other cellular functions [71]. It is believed that in general, resveratrol increases longevity in yeasts, mice, and several other organisms by activating Sir2 proteins, which, as sirtuins or class III histone deacetylase enzymes [72], regulate a variety of important cell-metabolic functions.

Although the manner in which sirtuins increase longevity appears to be highly complex, this regulatory effect of Sir2 on cell metabolism would resemble the effect of CR [73, 74, 81], and resveratrol has been found to induce patterns of gene expression similar to those in CR, although CR can extend longevity in the absence of Sir2 or other sirtuins [82–84]. These effects

require further study, including research on other organisms than those in which the effects of resveratrol have already been examined.

Anticarcinogenic Effect. Because of its capacity to prevent carcinogenesis at various points in its development by acting on molecular targets such as kinases, cyclooxygenases, reductase ribonucleotides, DNA-polymerase, resveratrol was designated a promising candidate agent for cancer chemotherapy [86]. In particular, resveratrol may exert an antineoplastic effect by decreasing the activity of cyclooxygenase enzymes, which increase blood and tissue levels of anti-inflammatory substances, reducing the risk of rectal and skin cancer by 30–40%. It may also counteract the development of cancer by decreasing the development of blood vessels in tumors, an effect also linked to the activity of cyclooxygenase enzymes. Beyond this is evidence that resveratrol stimulates the apoptosis of cancer cells by increasing the levels of various proteins including p53, p21, and p27 in such cells [87]. This protein-inducing effect has been found in cells of prostate cancer, lymphoma, and other cancers [88, 89]. A proapoptotic effect of resveratrol was also found in cultures of capan-2 and colo357 pancreatic cancer cells, with insignificant toxicity to normal cells of the pancreas [90]. A clearly antiproliferative effect of resveratrol *in vitro*, which increased when it was used in combination with other polyphenols such as curcumin and chrysin was found in cultures of the Caco-2 cells of human colorectal carcinoma [91].

Additionally, in preventing the transcription of nuclear factor-κB (NF-κB), resveratrol can act to prevent the wasting of skeletal muscle, cardiac trophy, and cachexia in cancer [92]. It has also shown a protective effect both *in vitro* and *in vivo* against colon cancer by increasing the apoptosis of neoplastic cells through the activation of p53-dependent pathways. In this setting, its effect is increased by the simultaneous use of grapeseed extract [93]. Resveratrol has also shown the ability under both experimental and clinical conditions to potentiate the antineoplastic action of cisplatin in colon cancer [94].

Clinical studies of resveratrol in oncology have begun. In the first phase of clinical research with resveratrol, a commercial formulation of resveratrol known as SRT501, consisting of micronized resveratrol, was given in a dose of 5.0 g/day for 14 days to patients with colorectal cancer metastatatic to the liver before they underwent hepatectomy, in a study designed to assess the safety, pharmacokinetics, and pharmacodynamics of the drug. The level of resveratrol in blood plasma after a single dose of SRT501 was 1942 ± 1422 ng/mL, which exceeded by 3.6-fold the plasma level after a dose of non-micronized resveratrol. In liver tissue, where the apoptosis of malignant cells increased by 39% following treatment with SRT501, resveratrol was detected at concentrations of up to 2287 ng/g [95].

Neuroprotective Effects. The studies described earlier in mice found that resveratrol promotes the elimination of amyloid plaque in brain tissue affected by Alzheimer disease. The sizes of affected brain regions decreased significantly, by 48% to 90%. Neither a significant increase in sirtuin levels nor signs of resveratrol were found in the brain in these studies. The investigators who conducted the study suggested that resveratrol exerted its effect against amyloid plaque by acting to increase cellular levels of cysteine, higher levels of which protect cells from oxidative damage and control the synthesis of amyloid precursor proteins (APP). An alternative explanation for this protective effect is that the cysteine induced by the action of resveratrol chelates copper or zinc in cells decreasing the concentration of perineuronal beta amyloid by as much as 49% [96]. In a case of Alzheimer disease, the use of resveratrol together with curcumin was found to improve cognitive function [99].

Data on the prospective treatment of Parkinson's and Alzheimer diseases with oligomers and dimers of resveratrol as well as with resveratrol itself [97] included a decreased level of β-amyloid peptide. In cellular models of Parkinson's disease, resveratrol was found to exert a protective effect against the rotenone-induced apoptosis of SH-SY5Y cells and to increase the

degradation of α-synucleins in PC12 cells by inducing autophagia in these cells. The inhibition of AMPK or SIRT1 or both was found necessary for this resveratrol-mediated induction of autophagia [98].

Antioxidant Effect. In addition to the mechanisms mentioned above, resveratrol appears to work through another mechanism to reduce oxidative stress in cells. In this, it promotes the activity of manganese superoxide dismutase (MnSOD), a group of enzymes that degrade metabolically generated superoxide molecules into oxygen and hydrogen peroxiode, thereby defending cells against damaging effects of oxidation [100].

Cardioprotective Effect. According to the so-called "French paradox," a culturally wide consumption of red wine, containing resveratrol and such other relatively strong antioxidants as quercetin and catechins, is responsible for a 4.5-fold lower incidence of cardiovascular disease in the French population compared with the British population despite its greater consumption of relatively fat-rich foods. Experimental and clinical research has found a positive effect of resveratrol on the cardiovascular system [101], with several possible mechanisms for this effect [102]. These include:

- A decrease in oxidative stress, inhibiting the oxidation of low-density lipoproteins (LDL)
- A reduced risk of vascular endothelial dysfunction through the activation of nitric oxide (NO) synthase, an important enzyme
- Inhibition of platelet aggregation

A cardioprotective effect of resveratrol was found in in vitro studies in which it was found to suppress the apoptosis of cardiac myocytes and reduce the content of caspase-3, an enzyme essential to apoptosis, and of various cardiac cytokines including B-type natriuretic peptide (BNP), NF-κB2, E-selectin, troponin, and tumor necrosis factor-α (TNF-α), in human myocardium subjected to experimentally induced ischemia and reperfusion [103]. Similar results were found in a Chinese study of male Sprague-Dawley rats, in which resveratrol, acting through the inhibition of Akt, a proinflammatory protein kinase enzyme, reduced cardiac apoptosis and the effects of shock induced by trauma and hemorrhage [104].

In a study in which rats which were fed three different doses of a biologically active dietary supplement (BAS) containing resveratrol together with 5% quercetin and 5% rice hull, the animals' hearts showed better cardiac performance and a smaller myocardial infarct size than did the hearts of animals fed a control diet of 5% quercetin and 5% rice hull alone when the two groups' hearts were studied after 1 or 3 months of either diet [105]. The study showed a hormesis effect (a positive influence of small doses on an organism while larger doses have detrimental effect) of pure resveratrol. It had cardioprotective effect in small doses and cardiotoxic effect in larger doses. Remarkably Longevinex had no hormesis effect—in the whole range of concentrations it was still a cardioprotective drug, even at the dose of 100 mg/100 g which in the case of pure resveratrol led to 100% of death of rats.

This same BAS also showed a cardioprotective effect when given to rabbits for 6 months. Cardioprotective effect of both resveratrol and Longevinex was detected during experiments on isolated heart with 2-hour reperfusion of the left ventricle after 30-minute ischemia [106]. Resveratrol has also been suggested for reducing the risk of cardiac toxicity caused by platinum-based antineoplastic drugs in elderly cancer patients, who are at greater risk than younger patients for myocardial ischemia and other adverse cardiac effects [107].

Anti-inflammatory Effect. Besides its other effects as described here, resveratrol is also an effective anti-inflammatory agent owing to such effects as decreasing activity of cyclooxygenase, a key enzyme in the generation of such pro-inflammatory cytokines as interleukin-17 (IL-17) and other pro-inflammatory substances [108]. A relationship of the antioxidative and

anti-inflammatory effects of resveratrol has been proposed as underlying a mechanism by which it acts through SIRT1 to upregulate antioxidant enzymes and downregulate NADPH-oxidase enzymes and TNF-a, exert an antioxidant effect on mitochondria, and markedly reduce inflammation and oxidative stress on the vascular system with aging, with beneficial effects on angiogenesis, capillary density, and regional cerebral blood flow. [109]

Clinical research has suggested an immunomodulatory effect of resveratrol itself and probably to an even greater degree with its metabolic glycoside derivatives [110].

Antidiabetic Effect. In animals fed a calorie-enhanced diet that increases the risk of type 2 or non-insulin-dependent diabetes (NIDDM), resveratrol has a number of positive effects, including:

- Increased sensitivity to insulin
- Decreased level of insulin-like growth factor (IGF-1)
- Increased activity of 5′-adenosine monophosphate-activated protein kinase (AMPK), an enzyme that stimulates oxidation of fatty acids by the liver, inhibits the synthesis of cholesterol and lipids, and triglycerides, stimulates fatty-acid oxidation in muscles, promotes muscle uptake of glucose, and modulates insulin secretion by the pancreas
- Increased number of mitochondria

A hypothesis according to which resveratrol can be useful in diabetic foot conditions by activating SIRT1 is based on its stimulation of increased insulin sensitivity, improved microcirculation and peripheral nerve function, enhanced angiogenesis, and increased production of cytokines [111].

Biological and possible therapeutic effects of resveratrol found in a number of experimental studies are shown in Table 4–2, with the reference for each study.

Reprogramming of somatic cells into pluripotent cells is one of the priorities of gerontology. In addition to a biotechnological approach to this, there is also a pharmacological approach. It is known that some pharmacological compounds can increase the efficacy of reprogramming of somatic cells into pluripotent stem cells (iPSCs), with this being particularly true of such inhibitors of the mammalian target of rapmycin (mTOR) as rapamycin itself and PP242; the sirtuin activators resveratrol and fisetin; the autophagy-inducing agent spermidine; the quercetin

Table 4–2. Biological Effects of Resveratrol

Effect	*Reference*
Antiproliferative action and proapoptotic effect	Creagh [112]
Decrease in level of IGF-1	Bashmakov [111]
Antibacterial and antifungal activity	Creasy and Coffee [114]
Antioxidant activity	Chanvitayapongs et al. [115]
Inhibition of free-radical formation	Belguendouz et al. [116]
Inhibition of lipid peroxidation	Frankel et al. [117]
Inhibition of eicosanoid synthesis	Kimura et al. [118]
Antiplatelet activity	Chung et al. [119]
Anti-inflammatory activity	Jang et al. [113]
Modulation of metabolism of lipids and lipoproteins	Frankel et al. [117]
Copper chelation	Belguendouz et al. [116]
Vasorelaxant action	Chen and Pace-Asciak [120]
Inhibition of activity of gastric H^+/K^+-adenosine triphosphatase	Murakami et al. [121]
Inhibition of protein-tyrosine kinase and protein kinase C	Jang et al. [113]

derivative LY294002, which is an inhibitor of phosphoinositide-3-kinase (P3K) LY294002; and curcumin [122].

Despite lack of clinical studies showing the effectiveness of resveratrol, if additional positive effects of resveratrol are considered, one can conclude that it has potential to become a multipurpose agent for therapy of several chronic diseases connected with aging.

In July 2011, the website ClinicalTrials.Gov [123] presented information about 36 clinical studies of resveratrol, some of them in progress and others completed. These included studies of resveratrol in type 2 diabetes, adiposis, Alzheimer's disease, and cancer. In one of these studies, a group of British investigators found small, but significant decline in the levels of insulin-like growth factor-1 (IGF-1) and IGF-binding protein 3 (IGFBP-3) [124] in 40 healthy human volunteers given resveratrol in daily doses of 0.5, 1.0, 2.5, or 5.0 g for 4 weeks, and concluded that this effect of repeated administration of resveratrol in high doses may retard the malignant transformation of cells and development of cancer [125]. Other studies have found a potentially important link between IGF-1 and an increased risk of colorectal, prostate, lung, and lacteal gland cancer, similarly suggesting a protective effect of resveratrol through downregulation of IGF-1 [126], and a decreased expression in monocytes of the genes for SOCS-3. NF-kB, SOCS-3, JNK-1, and IKKb, which play key roles in promoting dietary obesity, inflammation, and insulin resistance, among other functions, and an increased level of insulin substrate-1, which increases insulin sensitivity, with resveratrol at a dosage of 100 mg/day for 6 weeks [127]. Another clinical study, involving pre- and post-menopausal women given 36 g/day of grape powder per day during 4 weeks including 7 mmole/kg of resveratrol and 0,77 g/kg of quercetin showed a decrease of triglycerides (by 15% for pre- and by 6% of post-menopausal women), low-density lipoproteins and cholesterol (for 10% of pre-menopausal women). It is obvious that high concentrations of resveratrol in the majority of grape types and in wine has a role in so-called "French paradox" (i.e. inverse relation between level of cardiovascular diseases and wine consumption) [128].

In other studies, resveratrol was found to modulate cerebral blood flow, with the suggestion that its bioavailability be increased through conjunctive use of the alkaloid piperine [129], and that trans-resveratrol can increase blood levels of hemoglobin in the cerebral circulation and enhance mental capacity in comparison with a placebo among healthy young subjects solving a cognitive problem [130]. A completed clinical double-blind study found a dose-dependent effect of resveratrol at 250 or 500 mg/day versus a placebo in stimulating blood flow in regions of the prefrontal cortex during problem-solving [131].

According to the results of limited clinical research F. Zhang and Y. Wu [132] make a conclusion that use of resveratrol is promosing for prevention of ischemic stroke among aged patients.

The premises for testing resveratrol-based drugs in preventing carcinogenesis and treating different types of cancer is the capacity of resveratrol to protect DNA from damage and interfere with mutagenesis as found in preclinical researches The daily doses of resveratrol in such studies ranged from 250 to 500 mg, with higher doses of up to 5 g used in studies of derivatives of resveratrol such as SRT501 [133].

A study conducted by GlaxoSmithKline, Inc., investigated the safety and tolerability of SRT501 in a dose of 5.0 g/day for 14 days in patients with colorectal cancer and liver metastases of this cancer. Simultaneous with this research was carried out on the potential for using resveratrol in treating colorectal cancer at the University of Michigan Cancer Center. SRT501 has also been tested clinically against melanoma [134], and its tolerability at 5.0 g is being studied in patients with multiple myeloma [135]. Additionally, the first phase of a clinical study of the tolerability of resveratrol is underway at the U.S. National Cancer Institute (NCI) [136]. All of these studies have found a satisfactory tolerability of resveratrol and SRT501. No specific side effects of resveratrol, such a gastrointestinal upset or diarrhea were found at doses below 1 g in a small number of clinical trials of resveratrol that included analyses of its tolerability and

side effects [140–144], but these findings led to the recommendation that the maximum dose of resveratrol not exceed 1 g.

Several studies of resveratrol for preventing cancer in healthy aged people are being conducted at the University of Michigan Cancer Center, with results anticipated over a 10-year period. [137]

An ongoing Danish study is investigating potentially beneficial effects of resveratrol given at a dosage of 500 mg thrice daily for 5 weeks on obesity, metabolic syndrome, and inflammation in healthy male volunteers over the age of 18 years [138]. Thr Medical College of Wisconsin and U.S. Department of Veterans Affairs are conducting a study of the effects of resveratrol at 215 mg/day for 52 weeks in patients with Alzheimer disease [139].

The results of these studies, and of preclinical studies [145], seem highly encouraging as a basis for initiating wide clinical studies of resveratrol and its derivatives.

Polyphenols are the most common antioxidants in food product and are known to have a protective effect against cardiovascular diseases [147] and cancer [148, 149], as well as showing evidence of neuroprotective effects, such as in the case of Alzheimer's disease as discussed earlier [150, 151]. In addition to resveratrol, such other plant polyphenols as quercetin, butein, fisetin, piceatannol, and curcumin [146], all of which have been found to activate sirtuins [152, 153] and all of which have been found to inhibit cellular aging and to extend longevity in invertebrates.

Quercetin is an active component of many food and medicinal plants with significant, proven anti-inflammatory, antiproliferative [154, 155], and neuroprotective effects [156, 157]. Additionally, quercetin, like resveratrol and other polyphenols, also has antioxidant properties [165]. Quercetin and its analogues have been found to stimulate the apoptosis of human cancer cells *in vitro* through the activation of ERK or inhibition of Ras genes [158]. Among food-plant products containing substantial quantities of quercetin are capers, apples, tea, onions, citrus fruits, green vegetables, and most berries.

Among the most important known biological effects of quercetin in humans are antihypertensive effects, which have led to its use in metabolic syndrome, and improvements in endothelial function. It has been found to exert its anti-inflammatory effect and an antithrombotic effect by inhibiting the synthesis of various cytokines and nitrogen oxides, and this mechanism has also been considered to underlie both anti-infective and immunomodulating properties of quercetin [159].

Quercetin inhibits the metabolism of resveratrol, and may in this way potentiate the effect of resveratrol [160]. The combination of quercetin and resveratrol has been found to inhibit the aging of mouse adipocytes and to promote their apoptosis *in vitro* [162]. By itself, quercetin has been shown to inhibit quinone-reductase 2 (QR2) [163] and p56lck tyrosine kinase [164], and has been found to increase the longevity of *C. elegans* by 11% to 16% [161].

It is difficult to say whether quercetin has any important anti-aging effect due to the antioxidant properties that it has. Moreover, humans die not because of exceeding intense of molecular oxidation processes but because of the processes followed by cell hypertrophy, which is why clinical tests of antioxidants failed to demonstrate decrease of mortality. The anti-inflammatory effect of quercetin is based on a reduction in the levels of such pro-inflammatory cytokines as tumor necrosis factor alpha (TNF-a), observed in healthy people aged 20 to 40 years who consumed blueberry and apple juice, providing 97 mg of quercetin daily, for a month [166, 167]. Additionally, both quercetin and its derivative quercetin caprylate are considered to be activators of proteasomes, thereby exerting antioxidant and possible anti-aging effects. A anti-aging effect of quercetin was observed in fibroblasts of the HFL-1 cell line [168].

Butein, a biologically active plant phenol that is found in the stems of *Rhus verniciflua* Stokes, also appears to have anti-inflammatory [169], antihypertensive [170], and antineoplastic effects [171]. It may exert an antineoplastic effect in breast cancer by inhibiting the function of fibroblasts, which are thought to abet the growth of breast cancer cells (Samoszuki M, Tan J,

Chorn G. The chalcone butein from *Rhus verniciflua* Stokes inhibits clonogenic growth of human breast cancer cells co-cultured with fibroblasts (*BMC Complementary and Alternative Medicine* 2005;5(5) doi:10.1186/1472-6882-5-5)

Piceatannol is a hydroxylated analogue of resveratrol has long been used in Asia as a supplement and phytotherapeutic agent, and has shown anticarcinogenic properties [172, 173].

Other polyphenols do not have the same strong activating effect on sirtuins as does resveratrol, and do not activate genes considered to be potentially useful for inhibiting the aging of cells. However, at least two polyphenols, fisetin and butein, at a concentration of 10 mM in each case, have been found to prolong the survival of Saccharomyces yeasts, by 33% and 5%, respectively [174, 175]. Butein has been found to inhibit the mitogenic activation of the gene for mitogen-activated protein kinases 1 and 2 (MAPK 1/2) (extracellular signal-related kinases [ERK] 1/2 in hepatocarcinoma cells [176]. Fisetin decreases the mitogen-activated expression of the genes for MAPK (ERK) and p38 in human leukemia cell line (HL60) [177].

Curcumin is also considered a polyphenol with the capacity to interfere with cell aging [178, 179], and in experimental studies has shown effects resembling those of resveratrol. It blocks the pathway activating TOR, MAPKs, and Akt, as well as lipoxygenase and other enzymes important in cellular metabolism, and has been a focus of interest for its preventive and therapeutic potential in arthritis and other inflammatory diseases, cancers, and Alzheimer's disease, among others [180, 181]. Systems that enhance the transport of curcumin for cancer chemoprophylaxis, such as nanoparticles, liposomes, and microemulsions, were found to increase the antiproliferative action of this polyphenol [182].

The highest concentration of natural polyphenols is found in tea. Among its antioxidant polyphenols are catechines, flavonoids, theaflavins, and thearubigins. (Riemersma RA, Rice-Evans CA, Tyrell RM, Clifford MN, Lean MEJ. Tea flavonoids and cardiovascular health. (*QJM* (Oxford) 2001;94(5):277–282). Tea contains catechins in the form of water-soluble polyphenolic substances. Four basic catechins found in tea are: (−)-epicatechin (EC), (−)-epicatechin-3-gallate (ECG), (−)-epigallocatechin (EGC), and (−)-epigallocatechin-3-gallate (EGCG) [183]. EGCG, of which the highest concentration is found in green tea, is the most well-studied and most active catechin of those in green tea with regard to inhibiting carcinogenesis and decreasing oxidative stress. Although the mechanism of action of polyphenols in protecting health has not yet been determined several epidemiological studies have found significantly decreased incidence of prostate cancer in Asian populations that regularly drink green tea in larger quantity than do western populations.

A random, double-blind, placebo-controlled study examined the effect of capsules of a green tea extract with a full content of catechins for the chemoprophylaxis of prostate cancer in volunteers with prostate adenoma [185]. Sixty of these subjects were randomly divided into two groups, with those in the first group given 600 mg of green tea extract per day in the form of three capsules containing 200 mg each, with each capsule containing 5.5% EGC, 12.2% EC, 51.9% EGCG, 6.1% ECG, 75.7% total catechins, and <1% caffeine, while the second group of subjects received a placebo. Over the course of a year, only 1 of the 30 patients who received capsules of tea extract was diagnosed with prostate cancer, as opposed to 9 subjects in the placebo group who developed such cancer. These results are considered to be very promising. A study of cultured cells of the mouse hippocampal cell line H22, used as a model for oxidative stress, found that flavonoids protected these cells against injury by the glutamate-mediated accumulation of ROS, increased intracellular levels of glutathione (GSH), and protected the cells against an excessive influx of calcium ions, which causes cell death [186]. The study also found that the mechanism by which flavonoids protect against oxidative injury is highly specific for each of these substances.

A study of 30 flavonoids found that quercetin and fisetin had the capacity to maintain cell viability under conditions of oxidation stress [187]. Of the two, fisetin had the greater protective effect with regard to neuroglial cells in nerve cells in culture. Fisetin appears to affect

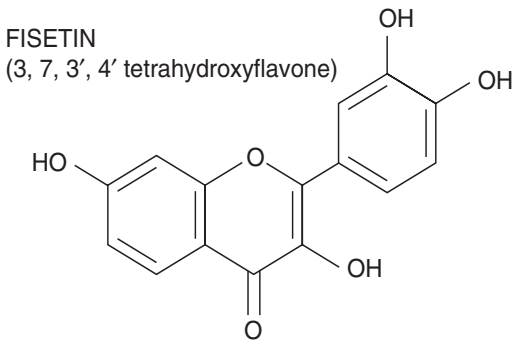

Figure 4–10 Structure of fisetin.

aging of the brain in a number of ways, including an improvement in neuronal function and survival by normalizing oxidation-reduction homeostasis, activating neurotrophic factor, and inhibiting inflammatory reactions by decreasing proteasomes activity and protein aggregation [188]. A notable feature of fisetin among the flavonoids is an effect by which it activates ERK and induces phosphorylation of the cyclic adenosine monophosphate (camp) response element binding protein (CREB) in rat hippocampus and facilitates long-term neuronal potentiation of rat hippocampal cells in vitro and enhances object recognition in mice, colectively demonstrating its ability to facilitate long-term memory [188]. Fisetin therefore appears to have the potential for use in an preventing age-related decline of brain function. Fisetin has also shown an antineoplastic effects in melanoma [189].

The highest level of fisetin (160 μg/g) has been found in strawberries [190], with five- to tenfold lower concentrations in apples and persimmon. Low concentrations of fisetin, whose bioavailability has not yet been studied, are present in kiwi, peach, grape, tomato, onion, and cucumber [191]. The poor solubility and bioavailability of fisetin make its medicinal use problematic. A solution to this problem can probably be found by optimizing its availability through pharmacologic or dosage-form modification, with a liposomal form containing P90G and DODA-GLY-PEG2000 having been found to improve its biopharmaceutical properties [192] and serving as the basis for its medicinal development.

Resveratrol is the only substance approved by the FDA as a supplement promoting longevity. More than 200 resveratrol-based supplements are made in the United States. Sirtris, Inc., a division of Glaxo-SmithKline, Inc., has been licensed by the U.S. Food and Drug Administration to investigate and develop potentially geroprotective drugs that work though the sirtuin SIRT1. Examples of such drugs are:

Longevinex: in capsules containing 100 mg resveratrol, 5% quercetin, and 5% rice hull

NuRev: in capsules containing 200 mg resveratrol, 50 mg of grapeseed extract, 5 mg of pomegranate extract, 25 mg of acai berry, and 25 mg of noni berry

Natural resveratrol: from red wine extract (*Vitis vinifera*): 200 mg; from *Polygonum cuspidatum* extract: 100 mg; from green tea extract (*Camellia sinensis*): 200 mg; from grapeseed extract (*Vitis vinifera*): 100 mg

Solstic Energy: Guarana grain extract (22%) (*Paulinia cupana*): 283 mg, red grape skin extract (*Vitis vinifera* L.): 50 mg; green tea extract (80%) (*Camellia sinensis*) (decaffeinated): 50 mg; Korean ginseng extract (3.5%) (*Panax ginseng*): 100 mg; vitamin B_1: 1.5 mg; vitamin B_2: 1,7 mg; vitamin B_3: 20 mg; vitamin B_5: 10 mg; vitamin B_6: 2.0 mg; vitamin B_{12}: 6.0 mkg.

Grapine with Protector:

Grapine (proanthocyanins from grape stones and redwood bark): 20 mg
Grape skin extract (*Vitis vinifera*): 50 mg
Vitamin C: 20 mg

Patent mixture: 200 mg
Broccoli (*Brassica oleracea*)
Carrot (*Daucus carota*)
Red beet (*Beta vulgaris*)
Rosemary (*Rosmarinus officinalis*)
Tomato (*Solanum lycopersicum*)
Curcuma (*Curcuma longa*)
Chinese cabbage (*Brassica rapa*)
White cabbage (*Brassica oleracea*)
Citrus bioflavonoids
Hesperidin
Calcium biphosphate
Cellulose (plant fiber)
Magnesium stearate (plant)
Silicon dioxide (silicon powder)

Production of concentrated nonalcoholic extracts of different types of red grape, generally of the cabernet type, is also well-established in the Commonwealth of Independent States. In Moldavia the product is known as "Immortelle," and in Ukraine as "Enoant" and "Vin-Vita." These grape extracts are produced from natural local materials such as grapeseeds, grape skin, and grape stems.

To conclude, to date clinical studies of resveratrol in humans have shown that it is well tolerated but has a low level of bioavailability. On the basis of pre-clinical studies of resveratrol that have so far been conducted, the chances that the first anti-aging drug will be made with it for human anti-aging therapy are very substantial [193].

4.4 mTOR AND RAPAMYCIN (SIROLIMUS)

Sirolimus, a macrolide antibiotic derived from the bacterium *Streptomyces hygroscopicus* discovered in the soil of Easter Island, is an immunosuppressant and antiproliferative agent used medicinally for preventing the rejection of transplanted organs and tissues, especially kidneys. Its trade name, "Rapamycin," originates from the indigenous name of Easter Island, "Rapa Nui." Although sirolimus was originally intended for use as an antifungal agent, its subsequently discovered immunodepressant and antiproliferative (antitumoral) properties and its capacity to prolong life of a number of organisms including mice and other mammals, even in older animals [194, 195] held the prospects for its use in prolonging human life.

The target of rapamycin in yeasts is designated TOR, and in mammals it is designated mTOR, or "mammalian target of rapamycin" [196]. It is also known as FK506 binding protein 12-rapamycin associated protein 1 (FRAP1)-protein, which is encoded by the gene designated *FRAP1*, and is a serine/treonin protein kinase regulating cell growth, proliferation, motility, survival, protein synthesis, and gene transcription. A proposed mechanism for the antitumor effect of rapamycin is presented in Figure 4–11 [197].

Figure 4–11 shows the signaling pathways that influence the *mTOR* gene and those resulting from its activation, including p70-S6 kinase 1 and eukaryotic initiation factor binding protein 1 (4E-BP1), which are the two major targets of the *mTOR* complex 1 (mTORC1) that results from activation of the *mTOR* gene. It is this complex that is inhibited by nutrient restriction and inhibition of growth factor, and that is important for the caloric restriction (CR) response and as potential targets for CR-mimetic agents [198]. Decreased levels of protein, which are related to the CR effect and are associated with increased longevity in experimental studies, are linked to the suppression of TOR in yeasts, *C. elegans* and Drosophila [199, 200].

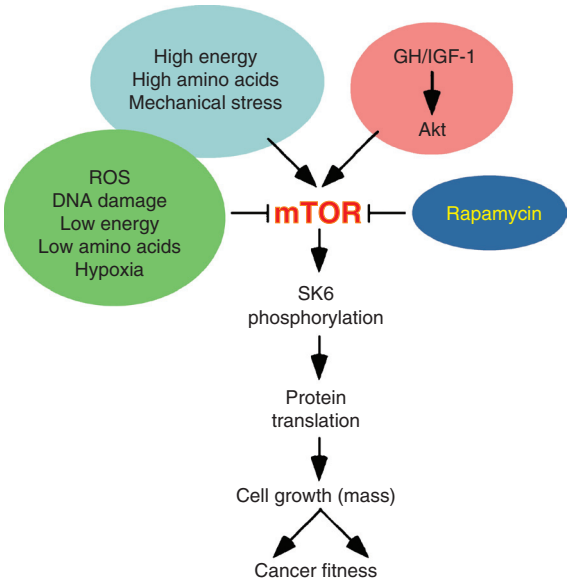

Figure 4–11. mTOR regulates cell growth, and its inhibition can be used to prevent cancer and decrease the activity of growth hormone (GH) and insulin-like growth factor (IGF-1).

The oral administration of rapamycin for mice over 20 months of age was found to prolong the lives of female mice by 14% and of male mice by 9% [201]. In a study comparing resveratrol, simvastatin, and rapamycin administered to mice after 9 months of age, only rapamycin was found to extend their longevity, by 10% for male and 18% for female mice [202]. In a study conducted as part of the Interventions Testing Program of the U.S. National Institute of Aging, genetically heterogeneous mice given rapamycin with food at the universities of Michigan and Texas and at the Jackson Laboratory in Bar Harbor, Maine, exhibited average extensions in longevity of 15%, 16%, and 7% (or an average of 13%) for female mice and 5%, 8%, and 15% (or an average of 9%) for male mice. The study suggested that rapamycin not only prolongs the life of aged mice but also decreases mortality among mice of "middle age," and that rapamycin inhibits cellular mechanisms related to cancer, which is the main reason for death in aged mice [203].

Other studies [204–206] have led to the hypothesis that rapamycin can prevent atherosclerosis, hypertonia, and hypercoagulation, thus preventing myocardial infarction and cerebrovascular accidents (CVAs), as well as osteoporosis, cancer, autoimmune diseases, arthritis, adiposity, diabetes, Alzheimer disease, and Parkinson disease.

A recent study at the Roswell Park Memorial Institute in Buffalo, New York, suggested that rapamycin suppresses a prosenescent phenotype in the Hutchinson-Gilmore progeria syndrome, a genetic disorder marked by accelerated aging and such aging-related features as athosclerosis and alopecia, and in which the mutant protein known as progerin is responsible for abnormalities in the cell nucleus and mitosis as well as for telomeric shortening [207]. The findings prompted the suggestion that rapamycin may not only be useful for treating progeria but also for normal aging. Although the potent immunosuppressant properties of rapamycin militate against its use as a geroprotector, it may be useful in lower doses, and newer analogues of rapamycin known as rapalogs may also prove useful for retarding aging [208].

4.5 BIGUANIDES AND METFORMIN

Research on mammals have shown that both hyperglycemia and hyperinsulinemia are important factors in aging and in carcinogenesis. It has also been suggested, as discussed earlier, that dietary caloric restriction (CR) decreases the level of IGF-1, which has been linked to various

mechanisms of aging and carcinogenesis [209]. This has started the search for geroprotectors among antidiabetic drugs as mimetic agents of CR and thus as geroprotective agents. Biochemically, CR achieves its geroprotective effect is through the activation of adenosine monophosphate (AMP)-activated protein kinase (AMPK) and inhibition of mTOR/S6K1, accompanied by an intense stimulation of the process of autophagy, in which cells destroy themselves through the release of lysosomal enzymes and related factors. In this context, it was recently demonstrated that the pharmacologically induced loss of ribosomal protein S6 kinase (S6K1), a target of mTOR, can have a life extending effect in mammals [210]. The biguanide family of drugs, and particularly metformin, are widely used in treating type 2 diabetes because of their inhibition of gluconeogenesis and promotion of increased insulin sensitivity, they can be considered typical CR-mimetic agents. They are being intensively studies for such a prospectively geroprotective effect.

It has been found in various studies that biguanides partly duplicate the effects of long-term CR in mice [211] and increase the average longevity of *C. elegans* [212], and that that the biguanides phenformin and buformin, as well as metformin, inhibit tumorogenesis and tumor growth [213–215]. Less is known about metformin as a CR-mimetic for healthy animals eating a normal diet, compared with numerous results in animal models of disease. An investigation of the long-term administration of metformin at 300 mg/kg/day to healthy male Fischer-344 rats given a standard diet [216] found that it increased longevity only insignificantly in comparison with that of control animals, and similar results were achieved in a study with 129/Sv mice given metformin at 100 mg/kg in drinking water, in which it insignificantly increased the average longevity of female animals, by 4.4%, and decreased that of male animals by 13.4% [217]. In this same study, metformin did not influence spontaneous tumorogenesis in male 129/Sv mice, but did decrease this by 3.5-fold in female animals, although the number of benign angioma increased slightly [217].

Clinical research has found that metformin decreases the risk of cognitive defects in patients aged 76 ± 6 years, and that the antidiabetic drug rosiglitazone potentiates this effect of metformin [218]. Metformin also inhibits the overexpression of human epidermal growth factor receptor-2 (HER2), through the AMPK-independent inhibition of p70S6K1 kinase activity, which is an effector of mTOR. Incubation with substances such as N-acetylcysteine, which suppress the production of active forms of oxygen (AFO), significantly increases the capacity of metformin to decrease the expression of HER2 [222]. This suggests that metformin may be useful for the prevention of breast cancer positive for the *HER2* oncogene. No connection has been recognized between this potential antitumor effect of metformin and any geroprotective effect of metformin. Spanish investigators have also found a metformin-induced inhibition of HER2 [221], and human studies have demonstrated that metformin works synergistically with the monoclonal antibody trastuzumab to eliminate cells of human breast carcinoma showing amplification of the *HER2* gene [219]. Studies of female mice transgenic for the *HER2/neu* gene demonstrated that metformin suppresses the development of breast carcinoma in these animals relative to control mice [220]. An important goal in studies of metformin as a geroprotective agent, especially for its combined use with small doses of rapamycin, is the determination of its minimally effective dose [223].

4.6 2-DEOXYGLUCOSE

2-Deoxyglucose (2DG) is an analogue of glucose and is absorbed by cells, in which it inhibits glycolysis. The result is an intracellular accumulation of 2DG and inhibition of the cellular metabolic processing of glucose, resulting in a decrease in cellular energy metabolism in a situation that appears to resemble that in CR [224]. Although 2DG inhibits

glycolysis, this does not appear to fully explain the cessation of growth of cells that take up 2DG. In preliminary studies, animals given 2DG as a food additive for animals have shown decreased in plasmic insulin concentrations and body temperatures resembling those with CR. A study of 344 male rats given 2DG as an 0.2% or 0.4% additive by weight to their food found a dose-dependent decrease in longevity rather than its prolongation [225]. It has been concluded that the pharmacological simulation of CR through the use of 2DG and the toxic effects of 2DG are separated by too fine a line to justify use of the latter substance in clinical research [226].

4.7 ANTIOXIDANTS

Antioxidants are compounds capable of interacting with active forms of oxygen and thus protecting a cell or organism from damaging influence of free radicals. For convenience, all substances having antioxidant properties can be divided into the following three groups [227]:

1. **Medicinal substances with antiradical properties (free-radical scavengers):**
   - 1.1. Endogenous compounds: α-tocopherol (vitamin E), ascorbic acid (vitamin C), retinol (vitamin A), β-carotene (provitamin A), ubiquinone.
   - 1.2. Synthetic medicines:
     - 1.2.1 Non-targeted antioxidants ("oxygen scavengers"): ionol (dibunolum), emoxipin (2-ethyl-6-methyl-3-pyridinol), probucol, dimethylsulfoxide (DMSO), olyphenum.
     - 1.2.2 Antioxidants (oxygen scavengers) targeted to mitochondria: SkQ, MitoQ is an artificial molecule—a quinone similar to CoQ1.
2. **Enzymes providing natural antioxidant protection and their activators:** superoxide scavengers, sodium selenite.
3. **Substances that block free-radical formation:** allopurinol, antihypoxants.

The hypothesis for using antioxidants as geroprotectors is based on the free radical theory of aging [228–230]. According to this theory, which also subsumes a wide range of age-related pathological processes (eg, cardiovascular diseases, age-related immunodepression and brain dysfunction, cataract, cancer and some others), free radicals formed through oxidative reactions in a cell or organism have a number of potentially damaging effects on nucleic acids and proteins that promote these macromolecules' degradation, causing aging, disease, or both [231]. In this process, molecules of superoxide ($O_2^{-}\bullet$), H_2O_2, the hydroxyl radical (HO•), and possibly singlet oxygen ($-O_2$), generally produced in the mitochondria of cells, cause damage to DNA, proteins, and lipids [232–234], and to structures made up of these substances, such as membranes, collagen, chromatin, and structural proteins. Such free radicals also damage substances that participate in regulating the expression of nuclear and mitochondrial genes, leading to aberrant cellular multiplication, cell death, irregular apoptosis, and other pathologic effects.

It has been determined that over a period of 70 years the human organism produces approximately one ton of oxygen radicals, even though only from 2% to 5% of the oxygen inhaled in air is transformed into toxic radicals. A rat cell can sustain up to 10^4 instances of DNA damage by active forms of oxygen, and under steady-state conditions up to 10% of protein molecules can undergo carbonyl-type modifications as the result of oxidation. Most of these effects are neutralized before they can damage any components of a cell, and only four superoxide radicals per million generated are not destroyed by enzymatic action. Endogenous sources of such antioxidant defense include various enzymes and vitamins (Table 4–3) superoxide scavengers (SOS), β-carotene, α-tocopherol, and uric acid in blood plasma [235].

Table 4–3. Antioxidation Substances

Target	*Agent*	*Function*
O_2	Superoxide scavenger	Transforms O_2 into H_2O_2
H_2O_2	Glutathione peroxidase	Transforms H_2O_2 into H_2O and O_2
	Catalase	Transforms H_2O_2 into H_2O and O_2
Free radicals	β-carotene (provitamin A)	Destroys liposoluble free radicals
	Vitamin E (α-tocopherol)	
	Vitamin C (ascorbic acid)	Destroys water-soluble free radicals
	Uric acid	
	Melatonin	Destroys lipid-soluble and water-soluble free radicals
Transition metals	Chelating agents	Prevents catalysis of free radical reactions by transition metals, ferrum and copper

Long-lived lines of *D. melanogaster* were found to have significantly more intensive expression of SOD, catalase, glutathione reductase, and xanthine dehydrogenase than did short-lived lines of flies [236].

Studies of transgenic lines of *D. melanogaster* with gene replication causing excessive activity of SOD and catalase support the free-radical theory of aging, finding that these flies live 20% to 40% longer than control flies and are significantly more tolerant of oxidative stress than flies without these genetic effects [237].

Vitamins

The two vitamins with antioxidant properties that have been best studied as geroprotective agents are ascorbate (vitamin C) and α-tocopherol (vitamin E). In 1984 Massie and colleagues [238] reported that adding 1% ascorbic acid (in drinking water) to the feeding of male C57BL/6J mice prolonged their lives by an average of 9% to 20% over that of control animals. Although repetitive experiments found a decrease in both lipid peroxygenation and oxidative damage to DNA in some tissues of mice given lifelong treatment with 0.4% ascorbate, they did not find any positive effect on longevity or biomarkers of cell stress (protein kinase enzymes participating in cytotoxic stress reactions) or inflammation. The study concluded that this was due to a tendency toward imbalance in both glucose metabolism and lipid exchange [239].

There is evidence that vitamin C by itself or in combination with other antioxidants can improve the phenotype of certain genetic models of age-related diseases. Lifelong administration of 0.4% ascorbate to mice with a model of Werner syndrome prolongs their lives [240]. Other studies have found a positive influence of vitamin C on the motor skills of mice with a model of Huntington's disease, and reduced spatial learning deficit among very old APP/PSEN1 transgenic mice with a model of Alzheimer's disease [241].

Beyond these results was the finding that a combination of N-acetyl-L-cysteine with vitamins C and E had positive effects on the function of beta-cells in diabetic C57BL/KsJ-db/db mice, with no such effects seen in aging of nontransgenic animals of wild type [242].

It therefore appears that vitamin C can have some positive effects on aging depending on mutations, genetic preconditions, and the types of age-dependent pathologies.

Besides alpha- and beta-tocopherol, the naturally occurring forms of vitamin E include gamma-tocopherol and delta-tocopherol. Alpha-tocopherol is the most effective antioxidant in the group. It is a lipid-soluble antioxidant present in all cell membranes, protecting lipids from oxidation,

and at least *in vitro* can also can act as a suppressor of singlet oxygen. Male mice given vitamin E in a dose of 5.0 g acetate alpha-tocopherol/kg throughout life had their median longevity increased by 40% and maximum longevity increased by 17%, and female mice given this regimen had a 14% median increase in median longevity but no change in maximum longevity [243]. Lifelong administration of alpha-tocopherol in a dose of 550 mg/kg to C57BlL/6 mice was similarly found to extend their median longevity [244], although lifelong administration of vitamin E at a lower dose of 400 mg/kg to BALB/c mice did not influence their median longevity [245]. In still another study [246], no positive effect of tocopherol on C57BlL/6 mice was found when its administration was begun after the age of 21 months.

Vitamin E added to food also did not influence the maximum longevity of C3H/He and LAF1 mice but did increase the number of mice that survived for 24 months [248]. This finding was also explained by a decreased number of spontaneous tumors among these animals, although another study found that the long-term administration of vitamin E with food to C57BL/6J mice did not influence the frequency with which they developed spontaneous lymphomas [249]. In a study of male Wistar rats given a diet of unsaturated fat as 15% safflower oil, the continued administration of vitamin E at 2000 mg/kg was found to protect against the early formation of cancers and to extend the animals' longevity by 50% [252]. A deficiency of vitamin E was not found to increase the frequency of thymic lymphomas in AKR mice [250]. Conversely, vitamin E was found to increase the incidence of carcinogenesis of the large bowel induced by 1,2-dimethylhydrazine [251].

It therefore appears that a positive effect of vitamin E depends on its dose, the age at which its administration is begun, and the genetic background of the individual to whom it is given. Porta and colleagues found that the addition of a high dose of α-tocopherol to food reduced the frequency and latency of development of spontaneously developing cancers but it did not influence the animals' maximum longevity [247].

Both vitamin C and alpha-tocopherol have shown beneficial effects in models of age-related disease. Thus, for example, the administration of vitamin E was found to impede cholinergic degeneration and the progressive deterioration of memory in mice with a model of Down syndrome [253], and vitamin E as well as the antioxiodants alpha-lipoic acid and acetylcarnitine inhibited the development of neurodegenerative changes in a rat model of Alzheimer's disease [254].

Vitamin E was also found to improve immune function and the apparent health of aged C57BL/6 mice infected with influenza virus [256], and can probably also increase insulin sensitivity [255].

N-Acetylcysteine

N-acetylcysteine (NAC) is an antioxidant and precursor of the natural antioxidant glutathione [257]. Several short-term studies have found a positive effect of N-acetylcysteine in rodent models of neurodegenerative and for cardiovascular diseases [258–260], and it was found to increase the life expectancy of mice with a model of systemic lupus erythematosus (SLE) [261]. Continuous administration of N-acetylcysteine at a concentration of 40 mM in drinking water was found to delay carcinogenesis in *p53*-deficient mice [262], and 5 weeks of the daily subcutaneous injection of N-acetylcysteine at 100 mg/kg was found to renew hematopoietic stem cell generation and reduce oxidative stress in cells of FOXO-deficient mice [263]. The continuous administration of N-acetylcysteine at 40 mM in drinking water was also found to inhibit the development of symptoms of premature aging in mice deficient in brain and muscle Arnt-like protein-1 (BMAL1), a transcription factor that regulates circardian rhythm [264]. More specifically, this same study found that N-acetylcysteine significantly increased the average and maximum life expectancies of mice and reduced their rate of age-dependent weight loss and the progression

of cataracts in these animals. However, it had no effect on the time of onset or severity of other age-related diseases, such as co-ossification of the joints, hair loss, or sarcopenia [264]. A recent study of N-acetylcysteine at 5 or 10 g/L of drinking water in genetically heterogeneous murine offspring of CByB6F1/J and C3D2F1/J parents found that its use from 7 months of age onward increased the life expectancy of male mice by about 40 % [264]. Unfortunately, the body mass of male animals declined significantly with the use of N-acetylcysteine, and their consumption of water also decreased in comparison with that of controls. It is not clear whether the antioxidant action of N-acetylcysteine or a CR diet caused these effects. Generally, however, N-acetylcysteine is not as effective as CR in increasing of life expectancy.

Catechins

The catechins are a family of plant polyphenols with antioxidant properties. Very few studies have been done of the long-term effects of catechins in mice not belonging to a specific genetic lineage; however, in mice of specific lineage it has been found, for example, that a daily intake of catechins increases the life expectancy of SAM-P8 mice [265] and delays memory loss in SAM-P10 mice [266, 267]. The SAM-P8-mouse shows many typical characteristics of mammalian aging including early memory impairment, defects in learning and behavior, mitochondrial dysfunction in muscles, cardiovascular dysfunction, cardiac hypertrophy, and reduced life expectancy. The SAM-P10 mouse shows accelerated aging of the brain, cerebral atrophy, and cognitive dysfunction. Among the catechins, epigallocatechin gallate has a neuroprotective effect expressed as inhibition of the progression of lateral amyotrophic sclerosis (ALS) in a mouse model of this disease [268, 269]. Epigallocatechin gallate from green tea was also found to reduce the symptoms of experimental nonalcoholic steatohepatitis induced by a high-fat content in the diet of Sprague-Dawley rats [270] and to prevents the development of diabetes in several rodent models [271].

Catechin was also effective in slowing the progression of diabetes mellitus and oxidative stress associated with this condition in Goto-Kakizaki rats [272]. Additionally, catechin was found to have a positive effect on homocysteine metabolism in mice with hyperhomocysteinemia [273], and to help slow the progression of atherosclerosis in alipoprotein E-deficient mice [273].

Alpha-lipoic Acid

Lipoic acid is a natural antioxidant with that can exhibit strong activity. It has been shown to effectively bind hydroxyl radicals, hypochlorous acid, singlet oxygen, nitric oxide, peroxynitrite, and hydrogen peroxide [274]. Studies with various rodent models of diabetes found a strong probability of improvement in cardiovascular and cognitive function, neurologic symptoms, and neuromuscular deficits with the administration of alpha-lipoic acid and dihydrolipoic acid [275]. There is reason to believe that these substances also have an anti-inflammatory effect. However, the use of lipoic acid begun in middle age in mice of different strains had no effect on life expectancy or carcinogenesis as compared with those of controls, and was therefore deemed to be less effective than CR.

Synthetic Antioxidants

The constant finding of products of the reactions of ROS with macromolecules in organs and tissues of the body suggests that natural antioxidant defense systems are insufficient to prevent damage to cells that are constantly exposed to oxidative stress, and that endogenous and exogenous antioxidants can play a significant role in geroprotection. Synthetic antioxidants have been found to increase the life expectancy of laboratory mice and rats as well as fruit

flies [276], and such substances with antioxidant properties as cysteamine hydrochloride or 2-mercaptoethylamine; 2,2-diaminoethylsulfide dihydrochloride; ascorbic acid; and 2-mercaptoethanol were found to increase the life expectancy of mice of different lines, but their effect was inconsistent and not always reproducible [277]. The most effective preparation in this regard was 2-mercaptoethylamine, which in one study increased the average life expectancy of C3H mice by 26%, and also significantly increased the time to progression of breast cancer, although the rate of occurrence of the disease was similar to that in control animals. Another antioxidant, hydroxylamine hydrochloride, reduced the frequency of spontaneous tumors in C3H mice but had no effect on their average life expectancy. Some of the antioxidant agents investigated augmented the longevity of AKR mice, but none had any effect on the progression of leukemia in these animals.

In other studies, the inclusion of 2-mercaptoethanol in the diet of mice increased both their average and maximum life expectancy, retarded the appearance of tumors, and reduced tumor progression [278, 279]. In a study of Swiss mice characterized by having a low frequency of spontaneous tumors, antioxidants had no effect on longevity [280]. Another antioxidant, dimethylaminoethanol [281], was also found to have no effect on the longevity or progression of spontaneous tumors in mice, although butylated hydroxytoluene (2,6-di-*tert*-butyl-4-methylphenol) increased the longevity of mice by reducing the frequency of their development of spontaneous tumors [282]. However, other studies found that both butylated hydroxytoluene and hydroxyanisole caused the progression of tumors in different locations in mice, rats, and hamsters [283, 284].

With regard to other antioxidants, the addition of 0.5% ethoxyquin antioxidant to the diet of 3-month-old C3H mice increased their life expectancy [285], although the investigators who made this finding did not provide data on the spontaneous occurrence of tumors in either treated or control animals. In rats, ethoxyquin caused preneoplastic changes in the kidneys and accelerated aging [286]. Epihyd (2-ethyl-6-methyl-3-oxypyridine), an analogue of vitamin B6 with antioxidant properties, had no effect on the occurrence or growth rate of spontaneous mammary tumors in mice of the SHK line, which is characterized by having a high frequency of such tumors, but increased of the time to tumor progression and the animals' average life expectancy. In another study, epihyd had no effect on the life expectancy or occurrence of spontaneous tumors in mice of the AKR line or F1 hybrids of C57BL x CBA mice [287]. It is possible that epihyd exerts an effect on the development of the mammary tumors through its antigonadotropic effect [288].

Retinoids

A significant positive correlation between the level of carotenoids in blood serum and brain tissue and the maximum life expectancy led to the suggestion that β-carotene and retinol, which are antioxidants and in some cases anticarcinogens, may have geroprotective properties [289]. Data supporting this assumption have only recently become available. When various retinoids were added to the feed of male rats ranging from 21 to 25 months of age [290], no difference was found in the average age at death, in survival, or in the occurrence of tumors of these rats and controls. Two of three retinoids tested increased the incidence of adenomas of the pancreatic islets, and one of them reduced the incidence of spontaneous tumors of the skin. In another study, β-carotene did not increase the life expectancy of mice [291].

Flavonoids

Flavonoids are the largest group of both water-soluble and lipophilic natural phenolic compounds. Catechins, which are polyphenols belonging to the group of flavonoids, are potent antioxidants,

and are responsible for the antioxidant properties of green tea. Epillocatechin gallate (EGCG), one of the catechin components of green tea, has more than 25 times the antioxidant potency of vitamins C or E. Just one cup of green tea daily has a more pronounced antioxidative effect than broccoli, spinach, carrots, or strawberries. The effects of flavonoids against cancer and cardiovascular disease have been investigated [292], and it has been shown that in C3H mice, the ingestion of EGCG slowed the growth of malignant tumors by 10% alone, and by 24 or 25% in combination with vitamin E. In these animals, an increase in apoptosis and decrease in the number of tumor-cell divisions were observed. However, the use of flavonoids against tumor progression in aged humans has shown mixed results [293]. More detailed study is needed to evaluate the effectiveness of different types of flavonoids for treating and preventing cancer.

Carnosine

Carnosine, or β-alanyl-L-histidine, has also shown protective effects under conditions of oxidative stress. In one study of middle-aged and older women it was found to prevent the accumulation of lipid hydroperoxides and protein carbonyls in the brain, to increase the activity of enzymes that ensure the maintenance of ionic and oxygen homeostasis of the brain, and to reduce the severity of neurological symptoms of Parkinsonism [294].

It is believed that carnosine can exert a geroprotective effect through the following mechanisms [295]:

1. Protection of proteins from glycosylation leading to protein cross-linking and the malfunction of cells. Glycosylation diminishes the activity of the vascular endothelial enzyme nitric oxide synthase (NOS), which causes hypertension and erectile dysfunction, and by damaging vascular collagen renders blood vessels less stable, as well as causing damage to collagen in the skin. Glycosylation of the crystalline lens of the eye causes cataracts, and in brain cells it leads to age-related deterioration of memory and degenerative diseases. Carnosine, as a "trap" for glucose, prevents such glycosylation, as well as activating intracellular proteasomes to digest glycosylated proteins and prevent cell death. It also protects tissues by increasing their insulin sensitivity and thus reducing sugar and insulin levels in the blood.
2. Protection of biologically important molecules and cells against oxidation by reactive oxygen species (ROS), the most toxic molecules that are generated in abundance within the body. Carnosine is called a superantioxidant because of its ability to neutralize any form of ROS, including oxygen free radicals (superoxide anion, etc.), non-radical ROS (hydrogen peroxide, etc.), and products of lipid peroxidation (peroxyl radicals, malondialdehyde).
3. Protection against brain injury by specific pathological proteins that are produced in Alzheimer's and Parkinson's diseases. Carnosine inactivates these proteins and prevents the formation of their products. Carnosine also improves brain function and prevents the onset of depression by reducing the activity of monoamine oxidase-B, an enzyme that oxidizes the neurotransmitters serotonin, dopamine, and norepinephrine, and thereby affects the conduction of nerve impulses and emotional states related to such conduction.

Selenium

Selenium, as a component of glutathione peroxidase, exerts an important effect against cell damage by lipid peroxides, prevents the autoxidation of lipid membranes, and is required for the normal absorption of vitamin E and the prolongation of its presence in plasma. The ability of

selenium to inhibit chemical carcinogenesis was found in rat models [296], and its introdunction to drinking water for a period of 15 months reduced the incidence of spontaneous mammary tumors in C3H mice by 72% [366]. In other studies, selenium in a dose of 2 or 6 ppm increased the average life expectancy of BALB/cfC3H mice and significantly reduced the occurrence of tumors in these animals [297], and increased the life expectancy of rats [298]. However, both the concentration of cholesterol in the blood and its deposition in the aorta increased, and at 30 months of age, the occurrence of tumors was significantly greater in treated animals than in controls. There is also evidence that liver tumors develop in rats that consume selenium in their diets [299]. An improvement in the immune status and life expectancy of aged mice was seen when zinc was added to their feed [300], but the incidence of tumors in control and experimental animals was not reported.

Supplementation of the diet of mice with an antioxidant mixture of β-carotene, vitamins E and C, rutin, zinc gluconate, and sodium selenite was found to increase their average life expectancy by 16% (but only when begun at 2 months of age), and increased the expression of several genes that participate in cellular antioxidant defenses.

So far, epidemiological data and the results of clinical trials of various antioxidants as means of reducing morbidity have not provided conclusive evidence of their effectiveness [301], save for vitamin E and possibly vitamin C, which reduce lipid peroxidation. The literature also indicates that many geroprotectors have undesirable effects, such as enhancing carcinogenesis in the colon in the case of α-tocopherol (vitamin E); increasing the incidence of adenomas of the pancreatic islets, in the case of beta-carotene and retinol; increasing cholesterol levels and enchancin the accumulation of cholesterol in the aorta, in the case of selenium; accelerating metabolism in the bones, in the case of growth hormone; and inducing hepatic tumors, in the case of dehydroepiandrosterone (DHEA).

Consequently, the results of studies of antioxidants for inhibiting aging and promoting rejuvenation are contradictory and ambiguous [302, 303]. On one hand, the available experimental data indicate that antioxidants can increase life expectancy and can have a positive effect on the course of degenerative diseases at different ages. This suggests that they may potentially be used to increase the duration and quality of life in humans. On the other hand, no sufficient clinical data exists to justify the systematic and long-term use of antioxidants in gerontology.

Antioxidants increase the average life expectancy (LE) and have little or no effect on maximum LE [304, 305]. Most investigators now agree that the benefit of antioxidants is due to their preventing the development of certain age-related diseases and thus improving the quality of life without slowing the aging process as such [306]. There may also be some adverse effects of high doses or the excessively long use of antioxidants. Thus, for example, a meta-analysis done in 2005 of 68 studies involving more than 232,000 persons [307] found that beta-carotene and vitamins A and E were associated with increased all-cause mortality. According to this study, the long-term intake of vitamin A increased mortality by 16%, that of beta-carotene increased mortality by 7%, and that of vitamin E increased mortality by 4%.

Nevertheless, a vast literature exists on the beneficial effects of plant extracts for the inhibition of age-associated diseases, and a diet with a high content of fruits and vegetables (ie, and antioxidant-rich diet) may be recommended for routine consumption. A recent randomized, double-blind clinical study found a polyherbal preparation made according to traditional Indian recipes to be safe and effective in restoring antioxidant effects in older subjects [308]. Rodent studies have provided considerable evidence for the positive results of fruit and vegetable polyphenols use. In one such study, rats fed for 8 months with a strawberry extract at 9.5 g/kg, spinach extract at 6.4 g/kg, or vitamin E at 500 IU/kg showed a delay in typical age-related changes in neurons and in cognitive impairment [309]. Further study showed that another 8-week regimen of extracts of spinach at 9.1 g/kg, strawberry at 14.8 g/kg, or blueberry at 18.6 g/kg body weight counteracted age-related cognitive impairment, although only the blueberry extract improved motor function [310]. A study of APP/PSEN1 transgenic mice

with a model of Alzheimer's disease found that antioxidant supplementation in the form of blueberry extract at 20 g/kg from 4 to 12 months of age normalized spatial memory in these animals [311], although it is likely that the effect of the blueberry extract was not due solely to its antioxidant properties but also to substances that increase the level of cell signaling and neuronal interconnection, or brain plasticity [312]. Similar results were obtained in 19-month-old Fischer rats given 8 weeks of a diet with a 2% strawberry content by weight [309]. It is particularly noteworthy that several polyphenolic compounds in blueberries can penetrate the blood-brain barrier in rats. Mass spectrometry has shown that these compounds localize in several brain regions including the hippocampus. The accumulation of polyphenolic compounds in the rat brain is probably due to the intensification of the cognitive functions [313]. Recent work with mice has shown that short-term (7 days) dietary supplementation at 60 mg/kg with an extract of wild blueberries expands their memory and cognitive function, and that this was associated not only with the presence of antioxidants in the brain, but also with the inhibition of acetylcholinesterase activity [314]. Another study showed that the positive effects of blueberries on spatial memory are at least in part the result of direct effects on hippocampal plasticity through increased neurogenesis [315].

Grape juice shows properties similar to the beneficial effects of blueberries, including potent antioxidant and anti-inflammatory properties and a positive dose-dependent effect of beneficial properties on cognitive and motor functions of aged male rats [316]. In the study in which this was seen, a 10% improvement in cognitive function was observed with 10% grape juice as a fluid, and improved motor activity in aged rats was obtained with a 50% grape juice concentrate. The investigators who conducted the study concluded that large quantities of polyphenols were needed for improving motor activity. The results of the study show that in addition to their known beneficial effects in cancer and heart disease, polyphenols present in foods may retard the aging process of neurons and prevent premature senility of motor function.

4.8 MITOCHONDRIAL ANTIOXIDANTS

The so-called mitochondrial antioxidant of V.P. Skulachev has a special position among potential geroprotectors—antioxidants. As discussed earlier, many researchers believe that aging causes a decline of physiological functions as a result of the effects of ROS-toxic derivatives of O_2 that are generated in the mitochondria, which are the main consumers of O_2 in the cell. Antioxidants directed at the mitochondria may therefore be inhibitors of aging, and the use of antioxidants specifically capable of penetrating the mitochondria, accumulating within them, and reducing the intramitochondrial level of free radicals may be a new stage in the development of geroprotective substances. It has already been shown that the use of lipophilic cations for the transport of bioactive molecules into mitochondria significantly increases their efficiency [317], and that 10-(6′-ubiquinol) decyltriphenylphosphonium (MitQ) in micromolar concentrations selectively blocks mitochondrial oxidative damage and prevents peroxide-induced apoptosis [390]. However, the difference between anti- and pro-oxidant concentrations of MitQ is not great [318], and the use of plastoquinone instead of ubiquinone was suggested for reducing its pro-oxidative activity [319]. The synthetic compound developed for this purpose was called SkQ1 (6′-plastoquinone) decyltriphenylphosphonium, and consists of plastoquinone as an antioxidant component, linked to a penetrating cation through a decanoic or pentanoic connector [320].

The hypothesis for this work was that such positively charged compounds specifically accumulate in the 2-nm-thick layer of the inner mitochondrial membrane, because these organelles are the only negatively charged compartments in living cells. In the case of SkQ1 the cationic, triphenylphosphonium portion of the molecule can serve as a transporter of antioxidants,

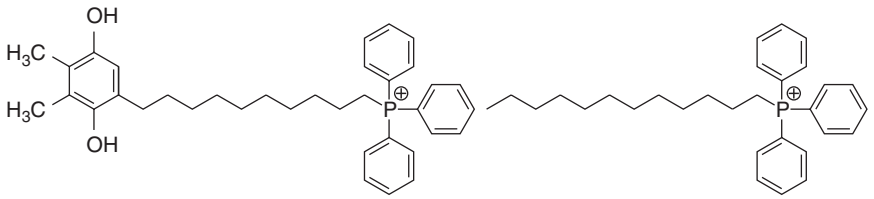

Figure 4–12. Molecular structural formulas of SkQ1 (left) and C12TPP (right).

and this has been confirmed in experiments with complex molecules such as the alkaloid berberine [321].

In the experiments of V.P. Skulachev on a flat, bilayered phospholipid membrane the following derivatives of SkQ were found to have the greatest penetrating capability: 10-(6′-plastoquinone) decyltriphenylphosphonium (SkQ_1), 10-(6′-plastoquinone) decylrodamin 19 (SkQR1), and 10-(6′-metilplastohinonil) decyltriphenylphosphonium (SkQ_3). The anti- and pro-oxidant properties of these substances, as well as of 10-(6′-ubiquinone) decyltriphenylphosphonium (MitoQ), were tested in aqueous solutions and in micelles, liposomes, isolated mitochondria, cell cultures, and whole organisms. Experiments with mitochondria showed that micromolar concentrations of these compounds had a pro-oxidant effect, whereas lower (nanomolar) concentrations exhibited antioxidant activity, which decreased in the order SkQ_1 = $SkQR_1$ > SkQ_3 > MitoQ [322, 323]. It was also shown that SkQ_1 can be regenerated through the respiratory chain of mitochondria, and is therefore a renewable antioxidant. Under conditions of oxidative stress, SkQ_1 specifically prevents the oxidation of mitochondrial cardiolipin. In cell cultures, $SkQR_1$, a fluorescent derivative of SkQ, stains only the mitochondria of cells. Extremely low concentrations of SkQ_1 and $SkQR_1$ block the H_2O_2-induced apoptosis of HeLa cells and human fibroblasts. Higher concentrations of SkQ were needed to protect cells from necrosis induced by ROS [324]. The experiments also demonstrated a high antimutagenic activity of SkQ_1 [325].

Experiments with fungi of the genus Podospora, the small crustacean *Ceriodaphnia affinis*, fruit flies, the fish *Nothobranchius furzeri*, and mice have shown that SkQ_1 prolongs these organisms' median life expectancy [326]. Another study [327] found that SkQ_1 in doses ranging from 0.5 to 2500 nmol/kg/day retarded aging of the reproductive system of outbred SHR mice and the manifestation of outward signs of aging, and decreased the animals' motor activity and endurance, but had no significant effect on carcinogenesis, increased average life expectancy and reduced mortality from non-tumor pathology. SkQ_1 also decreased motor activity in inbred mice of the 129/Sv strain, but had no significant effect on biomarkers of aging, life expectancy, carcinogenesis, or mortality from nonmalignant pathologies in these animals. Nor was any effect found for the studied doses of SkQ_1 on parameters of biological age, life expectancy, or the incidence of mammary-gland adenocarcinoma in transgenic mice carrying the *HER-2/neu* Gene.

In experiments with outbred mice, SkQ_1 was found to retard the age-related involution of thymic and splenic follicles, where T- and B- lymphocytes are formed [328], and in a model of Parkinson's disease, SkQ_1 had a distinctly positive antioxidant effect on mitochondria [329]. Among other findings for SkQ_1 are a therapeutic effect in mature retinopathy, and especially in congenital retinal dysplasia and cataract. Eye drops containing 250 nM of SkQ_1 restored the vision of 67 of 89 dogs, cats, and horses blinded by retinopathy. Eye drops containing SkQ_1 also prevented vision loss in a rabbit model of uveitis and restored vision to animals blinded by uveitis, as well as showing a beneficial effect in an experimental model of glaucoma.

SkQ_1 given to rats in advance greatly reduced arrhythmias induced by ischemia or H_2O_2. It significantly reduced the area of myocardium affected by myocardial infarction or insult, as well as preventing the death of animals with kidney ischemia. In *p53–/–* mice, SkQ_1 in a dose of 5 nM/kg/day reduced the level of ROS in the spleen and inhibited the onset of lymphomas in the same way as did N-acetylcysteine at concentrations a millionfold greater [330]. Other studies [331] showed that SkQ_1 and C12TPP—an analog of SkQ without the plastoquinone moiety—can operate as nanocarriers of fatty-acid anions through model and natural membranes. In this role they promote the uptake of fatty acids by mitochondria, leading to a mild uncoupling of respiratory processes and energy storage, reducing the mitochondrial membrane potential, and, consequently reducing the intramitochondrial production of ROS [332–334]. This may simulate the effect of CR.

The findings described here therefore suggest that compounds of the SkQ type hold promise as treatments for aging and aging-related pathologies.

4.9 SUCCINIC ACID

Succinic acid (butane diacid, ethane-1,2-dicarboxylic acid, $HOOC\text{-}CH_2\text{-}CH_2\text{-}COOH$) is a saturated dicarboxylic acid occurring in pure form as colorless crystals that are soluble in water and alcohol. It is present in small amounts in many plants and in amber. Succinic acid and its succinate salts and esters are universal intracellular metabolites that have drawn the attention of research investigators for their geroprotective potential.

The main pharmacological effect of succinic acid is the activation of aerobic glycolysis, an increase of oxidative processes in mitochondria, and increase intracellular levels of high-energy compounds [335, 336]. One side of the age reconstructions exchange is to reduce the oxidation of succinic acid, as indicated, and evidence of a decline with aging in the tissues of succinate dehydrogenase activity [338]. This decreases the oxidation of succinic acid, which significantly affects mitochondrial energy in old age. Because succinic acid has antioxidant properties, factors that promote its formation may be particularly effective in improving body function with aging. In studies with rats, the oral administration of sodium succinate at 300 mg/kg in 10-day courses with 1-month intervals for a period of 1.5 years beginning at 20 months of age increased both their average longevity, by 6.2% ($p < 0.05$) and maximum longevity, by 12.3%. The incidence of spontaneous tumors in animals given sodium succinate decreased by twofold and their multiplicity by 1.7-fold. A study of its long-term administration beginning at 3.5 months of age, found that succinic acid did not affect the average life expectancy of C3H/Sn mice, but increased their maximum longevity by 30.5%, as well as decreasing the incidence of spontaneous tumors by twofold [339].

Succinic acid, which is part of BAA Neuronol (Noogam), together with with melatonin and Epithalon, a peptide with the sequence Ala-Glu-Asp-Gly, is though to reduce carcinogenesis in SHR rats and in mice of the CBA, SHR, and SAM lines as well as mice carrying the *HER-2/neu* gene. In rat models of Alzheimer's disease, the dicholine salt of succinic acid given intraperitoneally for a period of 7 days improved cognitive function in an effect that persisted for 2 weeks after the end of treatment [340]. These findings prompted research into the effects of succinic acid in elderly people with manifestations of accelerated aging.

In a clinical study [341], essentially healthy people ranging from 60 to 74 years of age and showing symptoms of accelerated aging of the respiratory system were divided into a treatment group of 14 subjects that received succinic acid for 10 days at 0.5 g per dose given 3 times a day, while the control group, consisting of 12 subjects, received a placebo. The succinic acid regimen reduced the accumulation of lactate in the blood during hypoxic stress, and increased endurance and resistance to hypoxia.

4.10 NEUROTROPIC DRUGS

Decreased levels of catecholamines in the brain (chiefly in the hypothalamus) with advancing age, and abnormalities in the relationships of catecholamines to other biogenic amines, particularly serotonin, are considered important features of mechanisms of age-related change in the neuroendocrine system and ultimately of aging itself [342]. Reduced levels of catecholamines in the hypothalamus, achieved pharmacologically or by electrolytic destruction, was found to reduce the life expectancy of animals and to increase the frequency with which they developed tumors [343], whereas in rats, introduction of the neurostimulatory substance pentylenetetrazol reduced morphological changes in the brain with aging [344].

Among various findings relating to catecholamines was that dihydroxyphenylalanine (DOPA) in doses of 500 mg/kg prolonged the reproductive period and life expectancy of C3H/Sn mice [345]. The investigators who conducted this study did not provide data on the incidence of tumors in control and experimental mice, and daily dosing of female mice with DOPA at 60 to 90 mg/kg did not affect significantly the average duration of their life, but did increase longevity by 5.5 months, which significantly reduced the frequency of mammary tumors and increased the latency of their development.

Adding thiram (disulfide tetramethylthiuram, an inhibitor of dopamine β-dehydrogenase and other microsomal monooxygenases) to the feed reduced the incidence of spontaneous leukemia and tumors of the pituitary and thyroid glands in these animals but did not affect their longevity. Disulfiram, a close congener of the neurotrophic drug thiram and inhibitor of dopamine β-hydroxylase, reduced the incidence of spontaneous pituitary tumors in male and female rats and of mammary tumors in females [346].

Rats kept on a diet deficient in tryptophan, which results in a decreased level of serotonin in the brain, showed delayed aging of the reproductive system and of treated animals as a whole, as well as a reduced incidence of spontaneous tumors [347, 348]. When C3H mice were given the anticonvulsant diphenylhydantoin (phenytoin), which increases the levels of biogenic amines, especially dopamine, in the central nervous system (CNS), increased these animals' average longevity by 25% and reduced the frequency of tumor development 2.3-fold. On the other hand, diphenylhydantoin had no effect on longevity or the overall occurrence of spontaneous tumors in female rats, although it did reduce the incidence of their development of malignant tumors, slowed the aging of these animals' reproductive systems, and extended their cyclic estrous function [349].

DOPA and diphenylhydantoin prevent or retard the development of mammary tumors in mice and rats primarily through inhibitory effect on prolactin secretion, which plays an important role in the development of mammary tumors. Diphenylhydantoin also inhibits the secretion of insulin and glucocorticoids [350, 351]. However, the potential for use of diphenine as a geroprotective agent is controversial because of contradictory data on its carcinogenicity [352].

There is evidence that deprenyl, a known inhibitor of monoamine oxidase (MAO), prolongs the life expectancy of mice, rats, and dogs [353–355] and increases longevity in patients with Parkinson's disease [356]. It is believed that deprenyl also increases the activity of antioxidant enzymes, particularly SOD and catalase in the brain, which may play an important role in its geroprotective effect [357]. There is evidence that selegiline also inhibits the development of spontaneous and induced tumors in animals [358].

As noted earlier, the basis for research on the geroprotective activity of diphenylhydantoin (diphenine), dopamine, and deprenyl was their ability to stimulate dopaminergic systems of the brain that play important roles in mammalian aging. The mechanism of action of diphenylhydantoin on these systems is unclear. Dopamine is a source of catecholamines. Deprenyl inhibits monoamine oxidase B (MAO-B) and hence interferes with the inactivation of catecholamines. To date, only one report has provided information about an increase in life expectancy with

dopamine or diphenine, involving mice, and another publication has reported geroprotective activity for dopamine. Both studies were conducted a long time ago, and despite having promising results, were not further pursued for 30 years [359]. Only in the case of deprenyl did several independent research groups regularly find evidence that it can increase longevity. The most reproducible results with deprenyl were achieved with its administration to rats beginning in mid-life, at approximately 18 months of age. In aged rats, deprenyl was found to improve the immune system, memory, and learning ability, and to restore estrous cycles in females. The maximum increase in the longevity of rats given deprenyl was approximately 2 months [360]. Deprenyl was also found to increase the life expectancy of older dogs [361].

There is also evidence that acetyl-L-carnitine improves learning ability and motivation in rats [366].

Although research suggests that modifying the levels of biogenic amines in the brain may have promising geroprotective and antitumor effects, the only drug with proven benefit in elderly patients with cognitive and memory impairment and early-stage Alzheimer's disease is 5′-cytidine disphosphate-choline [362, 363]. However, some other commercially available drugs may prove effective for improving brain function with aging, and many investigators and clinicians consider psychotropic medications beneficial for improving mental function in elderly patients [368, 369].

Among commercially available drugs with effects on brain function are 2-(benzylhydrylsulfinyl)-L-acetamide, a recently developed stimulant designed to improve attention [364], and armodafinil 2-[(*R*)-(diphenylmethyl)sulfinyl]acetamide, an isomer of modafinil that is more active than modafinil in older people with narcolepsy, decreased memory, and reduced mental activity [365].

Ampacines, named from the neuronal ionotropic glutamate alpha-aminomethylisoxazol propionic acid (AMPA) receptor—with which they strongly interact—are a new class of nootropic drugs that promote brain activity with effects including increased focusing capacity, alertness, and improved memory and learning [367], are also used in aging persons.

4.11 GROWTH HORMONE AND OTHER HORMONAL PREPARATIONS

Ability to maximally maintain an active and quality lifes long as possible is crucial for healthy longevity. The list of hormones responsible for the biological effects of aging includes growth hormone (GH), insulin-like growth factor-1 (IGF-1), and dehydroepiandrosterone (DHEA), as well as melatonin, sex hormones, and thyroid hormones. Hormone-dependent changes associated with aging include the visceral deposition of fat, or adiposis, muscle weakness, osteoporosis, enuresis, loss of cognitive function, depression, and sexual dysfunction. At present time, both men and women live the last third of their lives with endocrine insufficiency. Hormone replacement therapy (HRT) is designed to overcome endocrine insufficiency and hinder or delay the development of at least some manifestations of the aging process [370].

Hormone replacement therapy with testosterone or growth hormone has been used for many years, beginning with its use in 1940 to treat testosterone deficiency in adult males. The recombinant hormone has been used since 1985. Both natural and recombinant testosterone are used as replacement therapy in natural aging of the human hormonal system.

4.11.1 Growth Hormone

The potential benefits of growth hormone (GH) in healthy older people include improving muscle strength [371, 372], quality of life [373], lipid profiles, and cardiac activity [374, 375]. A study by Rudman and colleagues [376] have showed that growth hormone therapy

restored the level of IGF-1 in persons over 60 years of age to that of persons 20 years age and reduced their body fat content by 14.2% in combination with an 8.8% increase in the muscle mass of the body [376]. In another study, the use of GH for 6 months in older, GH-deficient men and women reduced visceral fat and abdominal fat related to the risk of cardiovascular disease [377].

In a study of men 70 years of age and older, GH given for 6 months produced a modest increase in muscle mass, but this did not correspond with strength in the upper or lower extremities [379]. Lack of a clear anabolic effect of GH in reversing physical weakness in the elderly may be explained by insufficient dosing of or a limited duration of treatment. Moreover, despite the finding in some studies of increased muscle mass and muscle strength with the use of GH, these do not exceed what could be achieved with exercise [378].

Side effects of GH therapy include a dose-dependent retention of sodium and fluids accompanied by clinically significant edema, hypertension, carpal tunnel syndrome. and an increased level of glucose in fasting blood. The prolonged use of GH could theoretically also increase the risk of carcinogenesis. However, data about a relationship of GH to an increased risk of carcinogenesis remain ambiguous [380], and the safety of GH in elderly patients therefore remains unclear.

It is well known that muscle mass decreases and fat accumulation increases with age. To some extent this is due to an age-related decline in the production of GH that becomes evident after the third decade of life [381]. Reduced GH secretion is accompanied by a reduction in the producton of IGF-I by the liver. In rats, the transplantation of HG3 pituitary tumors producing GH was found to retard aging of the thymus [382]. These observations have led to a concept that age-related changes in the body are largely due to the decreased production of GH, which when compensated by the administration of exogenous GH will slow the aging process. When recombinant human GH was given thrice weekly for 6 months to a group of patients aged 61 to 81 years in an intramuscular dose of 0.03 mg/kg, their blood levels of of IGF-I and muscle mass increased and their body-fat deposits decreased [383]. Short-term administration of recombinant GH in a dose of 0.1 mg/kg/day to old male humans was accompanied by an increase in the oxidation of fat, increased nitrogen retention, and increased protein synthesis. Growth hormone replacement therapy also has a positive influence on bone metabolism [384], but it has also been observed that the administration of GH may accelerate the metabolism of bone and be accompanied by the development of carpal tunnel syndrome and gynecomastia [385].

Despite the enthusiasm of clinicians who use growth hormone in clinical practice, it should be used very cautiously. In rats, the long-term administration of growth hormone was found to substantially increase in the occurrence of tumors [387]. Some experiments with transgenic animals expressing genes responsible for the over production of GH in humans or animals have convincingly shown a reduction in longevity and high incidence of tumors [388, 389]. The potential of clinical use of pharmacologic GH replacement in antiaging therapy is believed to be high, but the adverse effects of such therapy have prevented its widespread adoption.

4.11.2 Thyroid Hormones

Neonatal administration of thyroxine to male rats induces a moderate postpubertal hypothyroidism accompanied by a 4-month increase in life expectancy, with this effect expressed to a lesser degree in females [390]. The report of these findings did not mention the occurrence of spontaneous tumors in control or experimental animals, but given that the levels of thyroid hormone in rats treated with thyroxine were decreased and prolactin levels were increased, an increased incidence of tumors in some locations, especially the mammary gland, might be expected.

4.11.3 Hormones of the Adrenal Cortex

Corticosteroids. Data relating to concentrations of glucocorticoids in the aging process are conflicting. It is known that their production increases with CR-induced slowing of the aging process [391], but it is also known that increased stimulation of the adrenal cortex (probably thnrough a weakening of catecholaminergic mechanisms of its regulation) also occurs with aging [392].

It has been found that the administration of prednisolone phosphate to a short-lived strain of mice with increased susceptibility to autoimmune disorders significantly inhibited their growth and increased their life expectancy from 1 to 2 years [393]. However, in experiments with long-lived lines of mice or with DBA/2J mice, which lack a predisposition to autoimmunity, prednisolone phosphate had no clear effect on average life expectancy [394, 395], nor did it change the incidence of spontaneous tumors in these mice. Apparently, the life-prolonging effect of prednisolone observed in the short-lived mice described above may be explained by its inhibitory effect on autoimmune processes that lead to the premature death of animals.

Dehydroepiandrosterone (DHEA). In recent years, dehydroepiandrosterone (DHEA), a natural metabolite of the adrenal glands that has a number of important biological properties, and like the other substances attracted the interest of researchers in gerontology. Dehydroepiandrosterone, a 19-carbon endogenous steroid hormone, is the major steroid secreted by the adrenal cortex, which produces 95% of this hormone in the body, and the testes, which produce 5%. Dehydroepiandrosterone (DHEA) and its sulfate form of dehydroepiandrosterone sulfate (DHEA-S) are weak androgens. The concentrations in of DHEA sulfate in the blood are 300 times higher than those of DHEA itself, and it has a long half-life and high stability that facilitate its use as a marker of adrenal androgen activity [396]. The content of DHEA sulfate in the body is also significantly higher than that of other steroid hormones, including glucocorticoids, even at their maximum concentrations under conditions of stress. Dehydroepiandrosterone sulfate has numerous biological effects: in peripheral tissues it is converted into testosterone or estrogens [397, 398], it is involved in the immune response [399], it has the properties of a neurosteroid, and it affects the state of the myocardium [400].

The finding that the production of DHEA sulfate decreases with age, and that in subjects 75 years of age its plasma concentrations had decreased to 80% of those at age 25 [401, 402], gave rise to the broad study of its geroprotective activity. In this, it was found that DHEA inhibits the synthesis of DNA and the formation of superoxide in tissues of the body, reduces body weight, and has antiatherogenic, antidiabetic, and anti-autoimmune activity [403]. Adding DHEA to the feed of NZB mice prevented the formation of autoantibodies to double-stranded DNA (dsDNA) and increased these animals' survival [404]. DHEA also prevented the development of age-related proteinuria and chronic nephrosis in mice of the S57BL/6 line and in rats, and increased these animals' longevity [405]. DHEA also inhibited the development of spontaneous mammary adenocarcinoma in C3H mice, as well as carcinogenesis induced by various agents in these animals' skin, lung, colon, thyroid, and liver. However, DHEA given to rats was found in some tissues to retard but in others to promote the neoplastic process induced by dihydroxy-di-n-propylnitrosamine [406].

DHEA, like other agents that work through the stimulation of peroxisome proliferator-activated receptor-alpha (PPAR), induces hepatomegaly, hyperplastic nodules, and hepatocellular carcinoma in rats [407]. The mechanism by which DHEA induces liver tumors is not clear, but is though to be mediated by oxidative stress.

There is general agreement that the administration of DHEA may have beneficial effects on the mood and well-being of humans with adrenal insufficiency [408, 409] and depression [410], and there is speculation that this therapy may have some benefit in Parkinson's disease and have the ability to improve cognitive function in older people [411]. It is also believed

that low levels of DHEA sulfate play a role in the development of Alzheimer's disease [412], which supports the hypothesis that it may improve cognitive function in old age. There is also evidence that DHEA acts as a neurotrophic factor with an antiapoptotic effect [417]. So far, however, clinical studies of DHEA sulfate have failed to provide convincing evidence in support of its hypothetical benefit on cognitive function with aging, No studies have been reported of the effect of DHEA replacement therapy in healthy elderly people, and there is no evidence that DHEA sulfate improves memory [413, 414], and according to at least one study, its usefulness in this regard is open to question [418]. There are even reports of a negative effect on memory with DHEA treatment [415] and a lack of benefit from its use in Alzheimer's disease [416].

A randomized, placebo-controlled study found that DHEA given orally as an additive to food had no effect on muscle growth, strength, or testosterone levels in middle-aged men [422], and a systematic review of the influence of DHEA on the physical performance of elderly people found that only 1 of 155 studies with DHEA reported improvement in a total score of physical characteristics, with the rest reporting no differences between the DHEA-treated and control subjects for each of the endpoints used in compiling the score [423].

Many studies have shown that DHEA has significant immunomodulatory, immunostimulating, and glucocorticoid-counteracting effects [419, 420]. A clinical study found that 6-month replacement therapy with 50 mg of DHEA given once daily, as compared with a placebo, improved glucose tolerance and reduced the plasma the level of triglycerides and of the inflammatory cytokines interleukin-6 (IL-6) and tumor necrosis factor-α (TNFα) [421].

The administration of DHEA for 6 months in a daily dose of 50 mg to 30 patients ranging from 40 to 70 years of age increased its blood levels in younger subjects within 2 weeks, in accompaniment with an increase the level of IGF-I. Two-thirds of the patients reported significant improvement in physical and psychological well-being [426].

Whether DHEA can be used as a geropreventive agent or perhaps as an alternative therapy for postomenopausal women with androgen deficiency syndrome remains uncertain [424]. In one study, the administration of DHEA to postmenopausal women produced a significant increase in the activity of natural killer (NK) lymphocytes [425]. However, an analysis of clinical studies [427] suggested that available data provide no basis for the use of orally administered DHEA in healthy postmenopausal women.

4.11. 4 Sex Hormones and Contraceptives

Testosterone. It is known that the serum testosterone level of men decreases gradually and in parallel with increasing age. Many manifestations associated with aging in men, including muscle atrophy, weakness, osteoporosis, reduced sexual function, and increased fat mass resemble changes associated with testosterone deficiency in younger men [427, 428], suggesting that a compensatory increase in the level of testosterone with aging can prevent or counteract the effects of aging [429].

In healthy older men with initially low-to-normal blood levels of testosterone, replacement therapy with this hormone has been found to increase muscle mass and decrease fat mass [430]. The increase in muscle mass and decrease in fat mass with the administration of testosterone to older men are comparable to the effects of testosterone given to young men with hypogonadism [431]. However, although muscle strength is a key factor in maintaining quality of life in older people [432, 433], most studies have found that increases in their muscle mass are not accompanied by increases in muscle strength or functional mobility. In the largest randomized, double-blind, controlled study of its kind so far conducted, testosterone in a daily dose of 160 mg orally for 6 months had no effect on cognitive function or bone density in older men with initially low testosterone levels, but did increase their muscle mass, although this was again

unaccompanied by an increase in muscle strength or functional mobility. With its promotion of muscle mass, the testosterone regimen simultaneously increased the sensitivity of insulin receptors in the enlerly subjects, and had no adverse effect on measures of prostate function [434]. However, a meta-analysis of clinical studies of the use of testosterone by healthy older men with low testosterone levels or clinical signs of hypogonadism found that they had a moderate increase in muscle strength together with an increase in muscle mass [435].

Besides decreasing body fat mass, testosterone replacement therapy in healthy older men is accompanied by a decrease in plasma glucose concentrations and increase in insulin resistance. Studies of testosterone in men with type II diabetes or adiposis showed a reduction in their blood glucose levels, plasma insulin levels, and blood levels of glycosylated hemoglobin, with an increase in insulin sensitivity [436, 437].

The decrease in body fat mass with testosterone replacement therapy is also accompanied by a reduction in total cholesterol, mainly owing to a reduction in HDL cholesterol. Exogenous testosterone increases the activity of hepatic lipase and enzymes involved in the catabolism of lipoproteins [438]. Data on the possibility of reducing the level of HDL are controversial. Two meta-analyses had different results in this respect, with one of them showing a slight, dose-dependent decrease in total cholesterol, HDL, and LDL with the intramuscular injection of testosterone in men with hypogonadism [439] and other showing that only the overall level of cholesterol was reduced after oral, intramuscular, or transdermal administration of testosterone to men with normal or reduced levels of endogenous testosterone [440]. In this latter study, insulin had no effect on HDL when administered intramuscularly and only a minimal effect after oral or transdermal administration. This may reflect the achievement of a higher compensatory serum level of estradiol with the intramuscular injection of testosterone, indicating an antagonistic effect of testosterone on lipase activity.

The intramuscular injection of testosterone moderately increased the bone density of the lumbar spine in hypogonadal men after 12 to 36 months of treatment, but did not change the bone density of the femoral neck [441], and failed to reduce the risk of fractures [442].

Epidemiological studies reveal a positive correlation between testosterone level and cognitive impairment [443, 444] and testosterone level and the incidence of Alzheimer's disease [445, 446]. Contrastingly, the findings in several clinical studies suggest that testosterone has no effect on cognitive function [447, 448].

Most studies of the quality of life with testosterone therapy do not provide grounds for discussing an improvement in this variable [450].

There is serious concern that men receiving testosterone therapy experience increased health risks from its adverse effects. Known adverse effects of androgens include gynecomastia, peripheral edema, and an increased hematocrit. However, the most important risk is that of the development or progression of prostatopathy, such as benign prostatic hyperplasia (BPH) and prostate carcinoma. A review of the risks of testosterone replacement therapy [451] and a study of its effects in men with late-onset hypogonadism [452] found no increase in prostatopathy, but some other reports have reported an increased risk of prostate cancer with such therapy [453, 454]. The use of testosterone in men with hypogonadism was found to increase levels of prostate-specific antigen (PSA) by an average of 0.30 ng/mL in young males and 0.43 ng/mL in elderly patients [455].

Summarizing these results we can say that the use of testosterone for replacement therapy in older men with normal or low-normal testosterone levels without clinical signs of hypogonadism is inappropriate. However, testosterone replacement therapy may be justified in older men with reduced testosterone levels regardless of the presence or absence of symptoms of hypogonadism.

At present, evidence for beneficial effects of testosterone in older men is insufficient, but the optimal target level of serum testosterone for achieving the greatest benefit and minimal risk of testosterone replacement therapy remains unknown.

Estrogens. The past half-century has seen the emergence of a belief, based on the findings in various studies, that estrogen replacement therapy during menopause can prevent many of the signs of aging, including osteoporosis, cardiovascular disease, and decreased sexual and cognitive function.

This belief subsequently led to the widespread use of hormone replacement therapy (HRT) for postmenopausal women. For more than 20 years, the American Academy of Anti-Aging Medicine (AAM) has supported the use of estrogen as a geroprotector, through a system of bio-identical hormone replacement therapy (BHRT).

The benefits of this therapy for women may include [456–458]:

- A reduced risk of osteoporosis and restoration of bone strength, and a reduction in vaginal dryness
- Maintaince of muscle mass and muscle strength
- A reduced risk of uterine and breast cancer
- A reduced risk of depression
- An improvement in sleep
- An improvment in mood, concentration, and memory
- An increase in libido

However, the enthuisiasm for estrogen replacement therapy declined sharply after publication of the results of studies conducted as part of the Women's Health Initiative (WHI) which found that such therapy increases in risk of breast cancer, pulmonary embolism, stroke, and coronary heart disease [460, 461].

The finding in recent large clinical studies of a detrimental effect of estrogen replacement therapy on the health of postmenopausal women has increased interest in the use of foods containing phytoestrogens as safe alternatives for reducing the severity of aging-associated cognitive impairment, neurodegenerative diseases, and other aging-related disorders [462].

In theory, phytoestrogens should have many of the typical estrogenic effects of estrogen itself but without the same risk of adverse effects. Numerous *in vitro* and *in vivo* studies suggest the potential for neuroprotective effects of phytoestrogens on the brain in conditions ranging from age-related memory loss to neurodegenerative diseases and cerebral ischemia. Although the most effective and safest phytoestrogens for this remain unknown, as do the optimal dosage and timing of dosing with such products, further clinical studies of the cerebral and neurologic effects of these compounds to brain seem warranted [463].

4.12 MELATONIN AND EPIPHYSEAL PEPTIDES

The epiphysis, or pineal gland, has diverse effects on the neuroendocrine system and on the metabolism and disposition of carbohydrates, lipids, and salt, as well as having immunomodulatory effects. Increasing data have been collected on the role of the epiphysis as the major pacemaker of the biological activities of an organism, operating like a "biological clock." It is known, for example, that light inhibits the production and secretion of the epiphyseal hormone melatonin epiphysis, whose maximum levels in the epiphysis and blood of humans and many species of animals therefore occurs at night, with the minimum occurring in the morning and afternoon. It is also known that epiphyseal function, like that of many of the endocrine glands of the body, decreases with aging, as manifested chiefly by a reduced scretion of melatonin and disturbances in the rhythm of its secretion. After the age of 60 years the difference in the daytime and nighttime concentrations of melatonin first declines to 80% of its peak lifetime level and then practically disappears [464, 465].

The question of whether a declining level of melatonin or changes in the rhythm of its secretion with age are a cause or an effect of aging has not been resolved [467], but it has

been suggested that because melatonin has high antioxidative activity it may be a means of retarding aging or countering the development of age-related neurodegenerative diseases such as Alzheimer's or Parkinson's disease [466–470].

A recent review [471] observes that melatonin replacement therapy has become common and is practiced around the world. Many experimental studies have also been done of melatonin as an geropreventive agent, including studies in which it was found that adding melatonin to the drinking water of rats significantly increased the life expectancy and blood testosterone levels of male rats [472]. In Swiss studies, transplantation of pineal glands of young mice to old animals increased their life expectancy by 42%, whereas implantation of the pineal glands of older rats in young animals reduced their lifespan by 29% [473, 474]. In other studies, the long-term nighttime administration of melatonin to mice not only increased their life expectancy but improved their immunocompetence, as well as increasing the size of their thymuses, adrenal glands, and testes and increasing the concentrations of testosterone and thyroid hormones in the blood [475, 476]. In another study, injections of melatonin restored immune function in both immunosuppressed and in aged rats [477]. Melatonin was also found to prevent the immunosuppressive effects of stress in mice [478].

Melatonin has also been shown to inhibit the apoptosis of cells of the gastric mucosa and of neuroglial cells in a model of Alzheimer's disease [479]. In another study, the administration of melatonin not only increased the longevity of mice but decreased carcinogenesis in the colon, mammary gland, and uterus of these animals [480, 481]. The administration of melatonin in drinking water to rats of middle age, to simulate the variations in the diurnal and nocturnal concentrations of melatonin inherent in young animals, reduced the mass of the rats' intra-abdominal fat. Interestingly, no changes were seen in hormone levels or the behavior of young rats given melatonin [482]. Some studies have shown a link between the effect of CR and those of melatonin [483].

The use of melatonin may also be justified from the standpoint of the free-radical theory of aging. Melatonin is one of the most effective antioxidants produced in the body, with a high efficiency in the binding of toxic hydroxyl radicals [484] that is several times greater than that of vitamin E [485]. Its antioxidant effects at the mitochondrial level may hypothetically render it a geroprotector [486].

When used in animal models of Alzheimer's or Parkinson's disease [487, 488], melatonin reduces the level of beta-amyloid and partly or fully prevents cell death. Melatonin was also reported to increase life expectancy but also the prevalence of tumors in female mice, prompting a warning of caution in its prospective use as a geroprotective agent [491].

In a clinical study [489] in which melatonin was given on a daily basis for 3 years to one of two monozygotic twins, this twin showed significantly fewer symptoms of Alzheimer's disease than were seen in the twin that did not take melatonin. Dietary supplements with melatonin were also effective in reducing the mild cognitive impairment that precedes dementia [490].

In accord with the manifold role of epiphyseal peptides in governing physiological functions [493], researchers in Russia found that the pineal extract known as epithalamin increased the life expectancy of mice and rats by 30% to 40% and inhibited the development of spontaneous tumors in these animals. In another study, epithalamin was found to restore sensitivity of the hypothalamic sexual centers to estrogen and a regular estrous cycle in aged female rats. Epithalamin also increased the duration of the reproductive period in rates, and extended their average life expectancy by 25%, and in some older female rats it restored the ability to bear offspring. Other findings in animal studies with epithalamin were that it inhibited lipid peroxidation, increased the activity of SOD and catalase in tissues, improved insulin sensitivity, decreased the hypothalamic threshold of sensitivity to inhibition by glucocorticoids, slowed the age-related decline in immune function, and increased the production of thymic hormones. In two lines of mice, epithalamin begun at the time of peak reproductive and immune function, of 3 to 3.5 months of age—or the equivalent of 25 to 30 years of age in humans—increased

longevity by almost a third. Experiments in which mice were given epithalamin beginning before the age of cessation of reproductive function also significantly increased their longevity. In all of these studies of the use of epithalamin, the increase in life expectancy was accompanied by a decreased incidence of tumors [494, 495]. Besides these findings, epithalamin was found to retard aging in fruit flies [496]. In clinical practice, epithalamin has given promising results for preventing premature aging and aging-related disease [497, 498]. Epithalon, a recently synthesized tetrapeptide analogue of epithalamin that also exhibits antioxidant effect [499], opens another prospective approach to the geroprotective use of such peptides.

4.13 IMMUNOMODULATORS

The immunologic theory of aging, in which the age-related decline in immune dysfunction reduces the resistance to infection and predisposes to the development of autoimmune diseases and cancer [500, 501], has prompted another avenue of research into geroprotection.

It has been established that the antitumor drug lenalidomide, an analogue of thalidomide, has a stimulating effect on T cells in healthy persons aged 21 to 40 years and those 65 years of age and older. At concentrations of 0.03 μM to 1 μM lenalidomide increased the T-cell synthesis of IL-2 by 17-fold and that of IFN-γ by threefold in the younger subhects, and inhibited the synthesis of IL-17. The same concentrations of lenalidomide in the older subjects increased the concentration of IL-2 by as much as 120-fold and that of IFN- γ by up to 6-fold, respectively, without inhibiting IL-17. Lenalidomide also inhibited the apoptosis of T-cells of elderly patients. These findings sugges that restoring T-cell-mediated immunity with low doses of lenalidomide can important in bolstering the immune response of elderly persons [503].

Studies are also being conducted of the use of recombinant cytokines to correct the balance of cytokines in aging organisms. These suggest that the stimulation of IL-2, IFN-γ (Th1 type) and IL-4 (Th2 type) by cultured spleen cells under the influence of recombinant cytokines could conceivably play a role in geroprotection [504]. At the same time, screening studies of inhibitors and activators of proteasomes are being conducted to find those that may be geroprotective. The finding that oleuropein, a compound found in the leaves of olive trees, has a beneficial effect on the aging of human fibroblasts supports the concept that proteasome activators may have potential as anti-aging agents [505].

In addition to this are studies of immunomodulators as geroprotectors. Studies with allophonic, or genetically chimeric, mice of the C57BL/6J-A/J, the original lines of which is differ in life expectancy, isoenzyme composition of glucose phosphate isomerase and H2 haplotype of lymphocytes (as markers), the overall survival was directly proportional to the percentage of lymphocytes in the peripheral blood of long-lived ancestors [506].

As a rule, factors that inhibit the immune system contribute to the development of tumors and reduce the longevity of animals [507]. For example, thymectomy in mice of the S57BL/6j line at 14 weeks of age significantly reduced their longevity [508]. However, in certain lines of mice, and particularly AKR mice, thymectomy or splenectomy performed immediately after birth or during infancy reduced the frequency of spontaneous leukemia and prolonged life, although a study of neonatal thymectomy in rats found that it did not increase longevity or the frequency of occurrence of spontaneous tumors [509].

Nor did the introduction of thymocytes or bone marrow cells from "normal" syngeneic mice to genetically dwarf mice characterized by a low life expectancy, hypopituitarism, and thymic hypotrophy increase their life expectancy [510]. However, injections of GH did increase the longevity of mice with a dwarfism gene from 5 months to 12 to 14 months. The investigators who conducted this study suggested that hormones determine the functional state of the thymus and of lymphocytes and so affect the aging process. In their view, the optimal maintenance of thymic and lymphocytic function through humoral factors can increase longevity in animals.

In other studies, injection of a suspension of syngeneic thymus tissue of young animals into aged mice had an varying effect on their life expectancy [511], while the transplantation of thymus glands from normal to autoimmune NZB/W mice increased their life expectancy by at least 1 month. [512]. After transplantation either of uncultured thymus tissue, cultured thymus epithelium or of thymocytes to NZB mice also increased their life expectancy, but only slightly [513]. Transplantation of the thymuses of 4 week-old AKR mice to 8-week- or 6-month-old syngenic mice had no effect on their life expectancy, although the transplantation of thymuses of 6-month-old to 8 week-old mice increased their mortality. Syngeneic The transplantation of thymuses of newborn C57BL/6 and BDF mice to syngenic mice, beginning when the latter were 2 months of age and continuing a 2 month intervals for a year, increased their average but not maximum life expectancy [514]. Transplantation of the thymus into adult mice had a weak effect.

There is evidence that several immunostimulants, such as levamisole, Bacillus Calmette-Guerin vaccine (BCG), and bestatin, influence life expectancy and the development of spontaneous tumors. In experiments on mice with a high frequency of spontaneous leukemia, the long-term administration of BCG increased their longevity but did not change their incidence of leukemia. In other studies the use of BCG reduced the occurrence of spontaneous leukemia and breast tumors, increased the latency of development of these malignancies, and increased life expectancy of treated mice [516]. Levamisole, which can restore immune function in aged mice, as well as the immunostimulant bestatin, increased the life expectancy of hybrid mice and reduced their incidence of spontaneous tumors. The immunomodifying agents azimexon and taftsin reduced the occurrence of spontaneous tumors in female C57BL/6 mice [517].

The detection, isolation, and synthesis of peptide hormones of the thymus that stimulate and modulae immune function has opened the prospect for new studies of the mechanisms of immunological aging and the development of measures to slow it, as well as new prospects for tumor immunotherapy. In recent years it has been shown that different peptide hormones of the thymus can improve the humoral and cellular immunity af aged animals. We have studied the effect of the thymic polypeptide thymalin, which was obtained and characterized by Morozov and Khavinson [518], on the life expectancy and development of spontaneous tumors in C3H/Sn mice. In this same study we evaluated the effect of epithalamin and polypeptide drug derived from the anterior hypothalamus of cattle. We found that this polypeptide drug increased the average longevity of mice by 20% and their maximum longevity by 2.5 months. At the same time, the administration of peptides from the anterior hypothalamus decreased the average longevity of treated mice by 1 month and their maximum longevity by almost 2.5 months. Treatment with thymalin or epithalamin significantly reduced the relative incidence of tumors in the mice. The most significant decrease, by 2.6-fold, was in the incidence of spontaneous mammary tumors upon treatment with thymalin.

In other studies, a synthetic peptide known as thymogen had a distinct geroprotective effect and reduced the incidence of spontaneous tumors in rats [519]. Another synthetic peptide, known as vilon, with the structure L-Lys-L-Glu, had a geroprotective effect in mice. The administration of vilon increased the physical activity and endurance of mice, reduced their body temperature, increased their life expectancy, and inhibited their development of spontaneous tumors. The long-term administration of vilon had no adverse effect on the development of treated animals. The available data for vilon indicate the safety of its chronic use and allow the recommendation of its use in clinical practice as a geroprotector and means of preventing age-related pathologies [520].

Also of interest are studies of the dietary supplements so-called "Transfer Factor" produced by "4 Life Research Co.", (USA) and represented as which is a natural peptide derived from cow colostrum and designated as an immunomodulator and adaptogen [521].

Thus, although the available data on the effect of immunomodulators on the life expectancy and spontaneous carcinogenesis are relatively small, this question is important and it is needed to continue studies of this important question in theoretical terms.

4.14 OTHER AGENTS AND FACTORS

4.14.1 Anti-inflammatory Drugs

Considerable experimental and clinical data indicate that chronic inflammatory processes with an autoimmune component are highly likely to develop with increasing age. A study of the aging of the cardiovascular system indicates an age-related increase in the level of hydrogen peroxide (H_2O_2) in endothelial cells of the carotid arteries, leading to activation of the transcription factor NF-κB [522], which is known to induce the activation of several genes that control inflammation. Inhibition of the mitochondrial production of hydrogen peroxide significantly reduces the expression of genes active in inflammation and reduces the mobilization of monocytes in models of inflammation in rodents. These findings indicate that the regulation of inflammatory processes may influence the development of age-related diseases. In this regard, several studies have shown that large doses of aspirin, an anti-inflammatory agent, may indirectly inhibit activation of the transcription of NF-κB. However, this inhibition is not the main mechanism by which aspirin reduces inflammation [523]. It was recently shown that the treatment of mice with aspirin at a dose of 58 mg/kg/day for 12 weeks alters their atherosclerotic phenotype [524]. In addition, the daily administration of aspirin at 30 mg/kg/day for 8 weeks to C57Bl/6 mice with a model of streptozotocin-induced diabetes restores their capability for learning and their memory capacity [525]. It has also been found that aspirin at 200 mg/kg/day promotes theapoptosis of tumor cells in mice with colon cancer [526].

According to data of the U.S. National Institute on Aging, aspirin increases the life expectancy of male heterogeneous mice but has no significant effect on females when treatment is begun at 4 months of age [527]. On the other hand, treatment with aspirin begun from 16 to 18 months of age had no positive effect on life expectancy [528]. Other anti-inflammatory drugs, such as nitroflurbiprofen, which like aspirin is a cyclooxygenase (COX) inhibitor, did not affect the life expectancy of genetically heterogeneous mice [529].

4.14.2 Procaine

The effect of procaine (Gerovital) on the lives of animals and humans has been studied for more than 50 years [530]. In experiments with rats, it was found to slightly increase the life expectancy of males but not of females. The investigators who conducted the experiments indicated a decrease (statistically insignificant) in the incidence of spontaneous tumors in animals treated with the drug. The mechanism by which procaine influences life expectancy remains unclear, but data about its weak inhibition of monoamine oxidase (MAO) activity in the brain are interesting [531]. However, a review in the Cochrane Database of Systematic Reviews [532] shows that the toxic effects of procaine in humans are stronger than the evidence favoring the probability of its preventing being effective for treating dementia and cognitive impairment. In short, its risks exceed its benefits.

4.14.3 Vitamins

The Cochrane Database also provides information about folic acid and vitamin B_{12} [533]. Here, a systematic review of randomized controlled trials done to determine the efficacy of folic acid and vitamin B_{12} for preventing and treating cognitive impairment in elderly persons concluded that there was insufficient evidence of a benefit with folic acid, with or without vitamin B_{12}, for preventing cognitive impairment or improving mood in healthy older people. However, in one

of the studies reviewed, which involved healthy elderly people with high homocysteine levels, an intake of folic acid at 800 μg/day for 3 years was associated with a significant improvement in both memory and the rapidity of processing of new information. Four of the clinical trials in the Cochrane Review involved subjects with cognitive impairments [533]. In one study of geriatric patients with Alzheimer's disease the administraton of folic acid at 1 mg/day significantly improved the overall response to cholinesterase inhibitors as well as the results of tests of activities of daily living and social behavior. Other studies involving patients with cognitive impairments did not show any improvement in cognitive function with folic acid with or without vitamin B_{12}. The review concluded that there is no strong evidence that folic acid with or without vitamin B_{12} has a beneficial effect on cognitive function in healthy or sick elderly people [533].

A research group at the Dartmouth Medical School in Hanover, New Hampshire, found that the recently discovered vitamin nicotinamide riboside (NR), a natural ingredient of milk, activates Sir2 in yeast, with a geroprotective effect. This finding helps in tracing the connection between caloric restriction (CR) and the geroprotective effects of sirtuins, in that the activity of Sir2 depends on the level of NAD^+, which is increased by limiting caloric intake. Nicotinamide riboside is a factor in the metabolic transformation of NAD^+ and can increase the cellular level of NAD^+ to thereby activate sirtuins without the need for dietary restriction [534, 535].

4.14.4 Coenzyme Q10 (Ubiquinone)

Coenzyme Q10 (CoQ10), or ubiquinone, is a compound synthesized in the liver and necessary for the cellular generation of energy. Chemically, coenzyme Q is a 2,3-dimethoxy-5-methyl-1,4-benzoquinone with an isoprene chain at position 6 of its benzoquinone ring. Research has clearly established a relationship between coenzyme Q10 as a carrier of electrons in the electron transport chain in mitochondria and as an antioxidant [536]. In mice fed diets containing coenzyme Q10 in a low dose of 0.68 mg/g or a high dose of 2.6 mg/g beginning at 4 months of age and continuing to 7, 15, or 25 months of age, the low dose of the coenzyme showed no effect on the animals' age-related decline in muscle strength or on learning or memory, while the high dose increased their spontaneous activity and retarded their age-related loss of visual acuity and abnormalities in spatial orientation and memory. However, coenzyme Q10 did not affect the survival of mice beyond 25 months of age [537].

The use of coenzyme Q10 as a geroprotector, on the basis of its antioxidant properties, did not have any effect under physiological conditions, but did increase the survival of irradiated mice [538, 539].

The findings made with coenzyme Q10 under clinical conditions provide sufficient grounds for its approval as a supplement to standard medical therapy for congestive heart failure [540] and Parkinson's disease [541], but for the greatest evidence for its benefit is in the treatment of age-related changes in the skin [542].

Dietary supplements containing coenzyme Q10 play an important role both in the prevention and treatment of several diseases and in protecting against the accelerated aging caused by inflammation and free-radical damage to cells and tissues [543]. Supplements containing coenzyme Q10 as well as vitamins C and E, alpha-lipoic acid, chromium, L-carnitine, and quercetin have demonstrated adjunctive benefits in diabetes, cardiovascular diseases, hypertension, congestive heart failure, age-related declines in brain function and vision, and other age-related health problems [544].

4.14.5 Inhibitors of Protein Biosynthesis

Inhibitors of protein biosynthesis, and particularly olivomycin, have been successfully used as geroprotectors [545]. Olivomycin selectively inhibits the DNA-dependent synthesis of RNA

by inhibiting RNA polymerase. It also has the properties of a chelating agent, binding some metal ions. Rats given olivomycin showed an increase in average longevity of 15.4% and in maximum life expectancy of 23%. Olivomycin significantly reduced the animals' serum and tissue lipid content and slowed and sometimes prevented the onset of age-related changes in several important functional and structural indicators. The study did not discuss the impact of olivomycin on the occurrence of spontaneous tumors.

On the other hand, the stimulation of protein metabolism by the chronic intraperitoneal administration of polyadenylic acid (poly-A) to CBA mice also slowed some manifestations of aging [546]. However, the mortality of mice treated with poly-A was slightly greater than normal and the occurrence of hepatocellular carcinoma and amyloidosis increased while its latency decreased.

When leupeptin, an inhibitor of protein biosynthesis derived from cultures of different strains of bacteria of the genus Actinomyces, was added in a content of 0.1% to the diet of male and female mice beginning at 3 weeks of age and continued for 480 days, 92% of the mice lived until the end of the observation period, as opposed to 83% of control animals. However, the incidence of spontaneous liver tumors in males given leupeptin was significantly greater than that in control animals, although the incidence of lung tumors did not differ [547].

Tritium oxide in a low dose was found to increase life expectancy in rats by 12.5%, with a simultaneous increase of 2.2-fold in the incidence of malignant tumors [548].

Studies reporting an increased life expectancy of animals with use of the biological-membrane stabilizers dimethylaminoethanol and meclofenoxate provided no data about the effects of these drugs on the development of spontaneous tumors [549].

4.14.6 Inhibitors of Crosslinking

The increase with age of intra- and intermolecular bonds in proteins, carbohydrates, and other biological components of living organisms is considered a possible mechanism of aging through its generation of defective macromolecules [550], even though the increase with age of such aberrant crosslinks has so far been demonstrated experimentally only for the extracellular proteins collagen and elastin, and possibly also for chromatin [551]. Restricting caloric intake, which increases the life expectancy of animals, delays the accumulation of collagen crosslinks [552]. Data on the ability of lathyrogenic agents to inhibit the formation of such crosslinks allow them to potentially be used as geroprotectors. Introduction of the lathyrogenic agent β-aminopropionitrile to the drinking water of mice significantly increased their longevity [553]. However, long-term administration of this drug to rats was accompanied by a decrease in body weight and a slowing of growth without a significant change in average life expectancy [554]. Male rats treated with β-aminopropionitrile also experienced some reduction in the incidence of benign tumors.

The proposed use of chelating agents (complexons) as geroprotectors is based on hypotheses about the importance of transition metals such as copper, zinc, and manganese in various processes of cross-linking [555]. These metals easily bind to active centers of many macromolecules, particularly enzymes, and may occasionally participate in the formation of coordinate covalent bonds as intramolecular and intermolecular crosslinks. Substances that prevent this or promote the elimination of metals from these crosslinks may prevent the formation of aberrant, aging-related macromolecules. The prolonged administration to female rats of one of the most widely used chelating agents, ethylenediaminetetraacetate (EDTA), together with their food and beginning on day 320 of their lives, increased ther average longevity [556]. However, although the administration of EDTA reduced the incidence of infectious and inflammatory disorders in the rats to which it was given, the incidence with which they developed tumors, which were chiefly malignant tumors, increased. The geroprotective effect of EDTA was significantly

dependent on the sex and age of the rats to which it was given and on when the drug was introduced into the diet. The finding of an increase in life expectancy with the administration of EDTA beginning in middle age, and complete absence of any such effect when it was begun in the presenile period, led the investigators who conducted the study to suggest that EDTA slows some age-related effects but has no effect on changes that have already occurred. In another study, the parenteral administration of EDTA had an insignificant negative effect on life expectancy of rats [557]. Other chelating agents, such as unitiol and penicillamine, had negative effect on longevity in rats [558]. Interestingly, in contrast to promotion of their excretion from the body, the addition of some metals, such as copper in the form of copper sulfate, to the diet of rats was found to increase their life expectancy and reduce the incidence of these animals' spontaneous development of mammary tumors [559].

4.14.7 Enterosorbents

Experimental data demonstrate a clear increase in longevity and slowing of the rate of aging of rats to which carbon enterosorbent was given in their feed [560]. However, how it affected development of tumors was not discussed. We have found that the introduction to the feed of mice of akvalen, a polyampholytic fibrous carbon sorbent characterized by its ability to effectively bind heavy-metal salts, carcinogenic polycyclic aromatic hydrocarbons, and nitroso compounds, increased their survival and inhibited their development of spontaneous tumors [561]. Considering the available data showing dietary fiber reducing the risk of cancer. Dietary fiber has enterosorbent properties, so it is quite possible that enterosorbents can adapted for use as geroprotective and anticarcinogenic agents.

4.14.8 Adaptogens

Adaptogens are a widely diverse group of medications of natural or artificial origin that can increase nonspecific resistance to a wide range of harmful physical, chemical, and biological effects, including stress and unfavorable effects in the external environment. The best known adaptogens and their groupings are:

- Botanical: *Rhodiola rosea*, ginseng, eleutherococcus, aralia (spikenard), astragalus, centaury, lemongrass, sea-buckthorn, ginger, etc.;
- Mineral: Shilajit
- Animal (including preparations of animal byproducts): reindeer antler (Cigapan), deer antler velvet (Pantocrinum), bee products (apilac, etc.);
- Synthetic trekresan (phenotropyl [phenylpiracetam], trekresan [*tris*-(2-hydroxyethyl) ammonium (2-methylphenoxy)acetate]).

Among the most studied adaptogens are preparations of ginseng and eleutherococcus. The range of their effects is extensive, and the adaptogenic properties of ginseng and eleutherococcus and their ability to protect and activate the genetic mechanisms of cells and affect the neuroendocrine system led to the suggestion of their use as geroprotectors.

Some researchers argue that only a few adaptogens (extracts of Ginseng, Eleutherococcus) can increase the average life span of rats to 10%, almost no effect on maximum life span [Chernilevsky V.E., V.N. Krutko. History of the Study of Prevention of life extension of aging. 2000. 3. :30-35.]. It has been suggested that ginseng can increase long-term resistance to stress and disease and therefore affect the lifespan. 270 mice of strain LACa were divided into three groups: one group which was given ginseng from 8 weeks of age, a second group

which was given ginseng from 52 weeks of age and an untreated control group. The mice were generally healthy. Their weights remained stable throughout their lifespan and were not altered by ginseng. Ginseng administration did not significantly alter the lifespan. However, ginseng did cause an exaggeration of the behavioural responses to mild stress. This effect was noticeable soon after ginseng administration and subsequently was maintained.270 mice of strain LACa were divided into three groups: one group which was given ginseng from 8 weeks of age, a second group which was given ginseng from 52 weeks of age and an untreated control group. Ginseng administration did not significantly alter the lifespan [562].

In a study of the effect of eleutherococcus on the occurrence of spontaneous leukemia in AKR mice [563], in which a liquid extract of the plant was given daily for 9 months in the animals' drinking water beginning at the age of 1 month, 57% of the treated animals developed leukemia, as compared with 73% of a control group. In another study [564], an extract of eleutherococcus given to C3H/He mice in their drinking water from the age of 2 to 2.5 months and continuing until the end of their lives did not alter their longevity or the latency of their development of spontaneous mammary tumors [564].

Available data are insufficient to allow a determination of whether adaptogens affect longevity or carcinogenesis, but data on the ability of eleutherococcus and ginseng to inhibit the development and metastasis of transplanted tumors and those induced by chemical carcinogens merit further study, as does the direct antioxidant effect of eleutherococcus [565].

4.15 CONCLUSION

In concluding a discussion of promising geroprotective agents, it should first be noted that different approaches exist for measuring the effect of any such agent on longevity, including those based on the average life expectancy (ALE) of all individuals in a population; the life expectancy of the youngest 10%, of 50%, and of the oldest 10% of a population; the maximum life expectancy in the population, and other standards. At present, a comparison of the effects of different geroprotective agents must be based primarily on ALE because the data for this are the most complete.

Three possible types of influence on the process geroprotectors retarding aging is shown in Figure 4–13.

The agent whose effects are shown in the uppermost plot (I) in Figure 4–10 extends the life expectancy of all individuals in the population, does not affect the rate of aging, and delays the development without affecting the incidence of tumors. The agent whose effects are shown in the middle plot (II) decreases the rate of aging and the occurrence of tumors. The agent whose effects are shown in the lowermost plot (III) does not affect life expectancy but increases the rate of aging and incidence of tumor development.

To date, it has proven impossible to increase the longevity of animals by more than one-and-one-half-fold. The recorded maximum increase in ALE in mice, of 49%, was obtained with the antioxidant dimethylaminoethanol [567], and most geroprotectors increase ALE by 10% to 20%.

It can be said that an ideal geroprotector should completely prevent an increase of probability of death with age, in which case the curve for mortality as shown in Figure 4–13 would be parallel with the abscissa, corresponding to linear passage of time. Most investigators agree that no claimed or prospective geroprotector has yet shown an indisputably positive effect in delaying aging or its effects though such geroprotector may be found in the future [568–570]. One of the reasons for this is the lack of objective measures for identifying them in a short experiment.

However, the possibility of extending life has been shown in many studies with living organisms and for many agents, including antioxidants, adaptogens, neurotropic drugs, monoamine oxidase (MAO) inhibitors, glucocorticoids, dehydroepiandrosterone (DHEA), sex hormones, growth hormone (GH), melatonin, pineal gland preparations, inhibitors of protein synthesis, antidiabetic

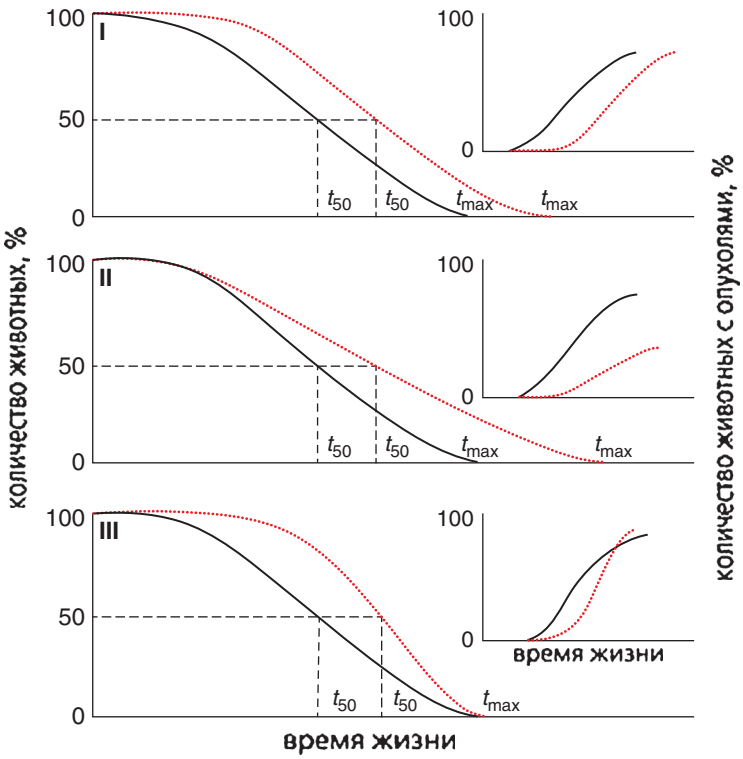

Figure 4–13. The effects on the longevity of animals and their development of spontaneous tumors of three types of geroprotectors (colored lines) [566]. The *x*-axes or abscissas of the plots show the ages of these animals and the *y*-axes or ordinates show the percentages of animals; the solid line represents controls and the dashed line the effects of the geroprotective agent; t_{max} represents maximum life expectancy and t_{50} represents half of the life expectancy.

agents, thymic hormones, immunomodulators, and enterosorbents, as well as mimetics of superoxide dismutase (SOD) and catalase. Many natural food supplements and synthetic medicinal and supplemental agents, especially some antioxidants, vitamins and hormones, have been actively marketed and used despite the absence of concrete scientific evidence of their effectiveness. This can be quite dangerous, since an excessive intake of antioxidants, hormones, or many other substances can imbalance sensitive mechanisms vital to the control of homeostasis. Among the reasons for this is the lack of a reliably established means for quantifying the effect of any such agent.

In the studies with animals in which a number of substances that would not be expected to increase longevity have shown a geroprotective effect, as in the case of radioactive dust and DDT, the reason for this has been hormesis, or the positive effect of small doses of certain substances that in large doses have adverse effects on the body [571]. Hormesis has been repeatedly demonstrated when substances known to be toxic, such as herbicides, pesticides, insecticides, hydrocarbons, ethanol, and organic solvents, amolng others, have been added to the nutrients or feed of different experimental plants and animals [572], which has raised the possibility of using such a hormetic effect in gerontology [573–575]. An explanation for such an effect, and the basis for its use in gerontology, may be the induction of a hormetic adaptive response in organisms exposed to stress.

Many investigators attribute life-extension by CR (the most effective proven geroprotection method) to hormetic effects [576, 577]. Many geroprotective agents have shown the typical U-shaped "dose–effect" curves when investigated, with no or even adverse effects at low levels, optimal

function when used in moderate amounts, and toxic effects with excessive quantities [578]. This effect has also been reported for the two most intensively studied geroprotective agents, resveratrol and rapamycin, and a similar U-shaped dose–effect curve has been repeatedly observed for antioxidants. For example, the SOD-mimetic agent Euk-8, when added in doses of 0.1 mM and 1 mM to the food of *D. melanogaster* deficient in the gene for SOD, significantly increased the average longevity of female flies as compared with that of controls, but significantly decreased longevity at a dose of 10 mM [579]. In epidemiological studies involving 34,492 postmenopausal women, the level of consumption of vitamin C and mortality from stroke had a U-shaped relationship [580].

Taking into consideration the results of these studies, it would seem reasonable to conclude that the effect of geroprotectors may be both specific and related to a direct effect on mechanisms of aging, and nonspecific, through hormetic effects of such geroprotective substances when the doses are in the hormetic range.

However, it is likely that agents or other interventions that delay the organism's genetic program of aging by normalizing hormonal, metabolic, and immunological changes would provide the most significant geroprotective and tumor-preventive effect. Among agents that appear to work in this way are the most promising simulators of CR, including resveratrol, rapamycin, antidiabetic biguanides, and probably also pineal gland extracts and melatonin. Antioxidants, antimutagens, enterosorbents, and other agents that block the action of damaging substances can serve as important additional means of preventing premature aging and development of tumors under high-risk environmental conditions. Some of these agents have already been successfully used in modern medicine, but further studies will help to increase their effectiveness and can open entirely new possibilities for their use. Clearly, such work will require the development of molecular biological, biochemical, and biophysical technologies, as well as time and funding.

At present development of new anti-aging technologies is rather slow despite significant technological advances in genetics and drug screening. Yet the basics of the biology of aging are understood sufficiently well for geroprotective technologies identified through studies with model organisms to be investigated in humans. Resveratrol and rapamycin, as well as metformin, all of which have effects resembling some aspects of caloric restriction (CR) and are directed at prolonging life, represent the first steps in this translation process. All compounds have been found to slow the aging of yeast and invertebrates and to increase the longevity of rodents. All compounds have also shown impressive effects in rodent models of age-related human diseases. Clinical trials have begun of resveratrol as an agent for treating cancer, and rapamycin has already been approved for clinical use. As was recently stated [581], the results of studies with these compounds are very encouraging in terms of creating the first drugs for treating age-related diseases in humans, and most importantly, of modulating the molecular mechanisms of human aging.

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Chapter V

The Gastrointestinal Track as a Complex Neuroimmunoendocrine and Metabolic Organ. Microbiota of the Gastrointestinal Tract: Role and Applied Aspects in Gerontogenesis

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5.1 NEUROIMMUNOENDOCRINE COMPONENT OF THE DIGESTIVE TRACT

The digestive tract is represented by a complex system of regulation. The nervous system of the digestive tract is the most extensive network of nerves outside of the central nervous system and its immune system is the most extended. Important interactions occur with the intestinal microflora. In addition, polypeptides and amines play an important role in humoral regulation of digestive functions. The source of these compounds are neuroendocrine cells of the mucous coat of the gastrointestinal tract that make up the diffuse endocrine system (in 1969, E. Pierce suggested the term amine precursor uptake and decarboxylation [APUD] system). Unlike the cells of the closed glands, these cells are not organized in the glandular structure but instead are among the other cells of the mucous layer along the greater part of the gastrointestinal tract. Most of

the neuroendocrine cells of the gastrointestinal tract are located in the stomach, small intestine, and pancreas—a small quantity can be found in the esophagus and large bowel as well [1, 2].

It is known that neuroendocrine cells differentiate all the time, thereby complicating their constitution, and they migrate from the crypts to the tops of the villi. This separation and migration provides a coherent response to the contents of the digestive tract, which come in contact with a wider surface of the mucous coat. Due to the short lifetime and sufficiently rapid inactivation of polypeptides produced in the liver or directly in the bloodstream, their effects on organs outside the digestive system are decreased [3].

Another component that regulates a number of important functions in the body, especially in the digestive tract, is human microbiota [4]. Research of the digestive tract's microbiota is still needed, even though a large number of scientific works have been published. These studies are of particular relevance to elderly patients, considering the frequency of occurrence of diseases of the gastrointestinal system which then increase manifestations of pathological aging. It should be borne in mind that the intestinal epithelium features both high production of cells and well-defined tissue architecture. In general, the lining of the gastrointestinal tract consists of several cell types with different proliferative properties. Thus, mucosal tissue is composed of a relatively small number of stem cells, a layer of cells other than stem cells but of a high proliferative activity, neuroendocrine cells, and a large area of differentiated cells that migrate into the lumen [5].

It is known that intestinal epithelium undergoes changes with age and this is essential for understanding how the microbiota functions in the process of aging because the digestive tract's microbiota acts as a "metabolic organ." Knowledge of the digestive tract's microbiota has been established recently as a meaningful part of the immune and neuroendocrine systems of a microorganism (the so-called balanced microbiota in health), which allows for new applied research on the modulation of microbiota for therapeutic purposes in aging. For example, it has been revealed that the toll-like receptors (TLRs) and nucleotide-binding oligomerization domain containing 2 (NOD2) receptors are key mediators of the local immune system in the intestinal mucosa and are involved in maintaining the homeostasis of the mucosa and symbion. In health, the TLRx signalling protects the epithelial barrier of the intestine and provides tolerance to the symbion whereas the NOD2 signalling ensures antimicrobial activity and prevents pathogenic invasion. In disease, the aberrant TLRx and/or NOD2 signalling may stimulate the extreme inflammatory responses that leads to an acute or chronic inflammatory bowel disease and other diseases typical to aging organisms [6].

According to the literature, over the human body surface, there reside various microorganisms specific to the habitat and the number of microbial cells exceeds the number of macroorganism's cells by an order of magnitude. The large bowel contains 10^{11} to 10^{12} and more of microbial cells per 1 ml; this extremely high density of genes is double the number of genes in the human genome. Recent studies have identified 9 million unique microbial genes in the human intestinal canal. Co-evolution with this bacterial ecosystem is essential for the macroorganism for digesting nutrients from food, resisting the colonization by pathogens, stimulating proliferation of the enteric epithelium, and regulating adipopexia. The important role of microbiota has been proven in vitamin synthesis, degradation of xenobiotics, metabolism of bile, and macroorganisms' hormones. Moreover, the pathogenesis of several diseases, including type 1 diabetes, inflammatory bowel disease, allergies, adiposity, and cancer of the stomach and colon, is related to the disbiosis of the microbial colonization. A macroorganism's metabolic activity is significantly affected by microbiota and this direct influence on the phenotype of mammals has brought about the term "ecological development" and the definition of mammals as "super organisms" [7].

The *Human Microbiome* project by the U.S. Institutes of Health opened a new vista in the scientific research of human intestinal microbiota. The human digestive tract is populated with 100 trillion bacterial cells whose collective genomes and microbiomes reflect the

evolutionary selection acting both at the macroorganism and the microbial cell levels [8]. Recently, the opportunities of employing metagenomics-based techniques have proven to be highly appropriate for deeper examination of the biological variety of the human intestinal microbiota. The human endogenous microflora is a determinant "authority" in regulating the development of the intestinal epithelium, local immunity, and digestion and plays a fundamental role in maintaining health and during diseases. Microflora has been proven to protect epithelial cells from damage, regulate the macroorganism's adipopexia, and stimulate the intestinal angiogenesis [9].

Many authors note that studies of the human genome have improved our understanding of microbial pathogens and the symbiont, and vice versa. Potential criteria have been suggested regarding the use of a macroorganism's gene in microbial pathogenesis [10]. In this aspect, it would be vital to introduce the "second project of the human genome." Thus, there appeared a bulk of new targets affecting the function of the gastrointestinal tract, and it especially those changes that occur in the microbiota as a result of the human aging. In this regard, we consider it promising to conduct scientific research in the field of specific biomarkers that reflect the status of the microbiota and the intensity of the macroorganism's response at the level of the neuroendocrine and immune systems.

5.2 THE INTESTINAL MICROBIOTA: CHARACTERISTICS IN SENIORS

Microbiota is a common term for a collection of microorganisms such as bacteria, fungi, protozoa, that inhabit the human alimentary tract in a normally symbiotic relationship. When analyzing the neuroendocrine, immunological, and physiological aspects of this symbiosis, it is important to note the dynamic relationship of the microbiota and the human macroorganism in the processes of developing and aging. The disruption of the symbiotic relationship within the microbiota-macroorganism system results in a condition called dysbacteriosis.

The length of the gastrointestinal tract of an adult is about 7 meters on average (in some cases, up to 20 meters) whereas the population of organisms inhabiting the bowel reaches 10^{11} cells per gram of feces [11].

It is considered that the number of microbial cells is 10 times greater than the number of its own, and the total number of genes of microorganisms that inhabit the large intestine is 100 times greater than in the human genome [12]. The majority of microorganisms present in the gastrointestinal tract have not yet been cultivated or identified [13]—as we know, only 10% to 15% of the microorganisms inhabiting the human macroorganism have been cultivated [14, 15]. Nevertheless, the gastrointestinal microbiota are directly involved in the life of a macroorganism and affect its health [16].

Evolving together with a macroorganism for millions of years, the intestinal microbiota contribute significantly to human physiology; in particular, they play a great role in the processes of digestion, metabolism of endogenous and exogenous compounds, participate in immunological defense mechanisms, and prevent colonization of the gastrointestinal tract by pathogenetic microorganisms. Microbiota reflects the condition of the macroorganism responding to physiological, nutritional, climatic, and geographical factors by changing its qualitative and quantitative composition [17]. Intestinal microflora features a certain stable composition called a microbiota kernel [18].

According to preliminary data, the organisms composing the human microflora belong to one of three types of organisms. Each enterotype is determined by a dominant kind of microorganism and has an appropriate name such as *Bacteroides, Prevotella,* and *Ruminococcus*. For example, the *Bacteroides* enterotype is distinguished by its ability to decompose carbohydrates as well as promote production of vitamins C, B2, B5, and H. On the contrary, *Ruminococcus*

enterotype improves absorption of carbohydrates and raises blood sugar levels. They synthesize folic acid and vitamin B1. *Prevotella* destroys, in the process of life activity, the protective mucous sheet, which probably predisposes it to prolapses in the intestinal mucosa. Consequently, an enterotype can reveal the peculiarities of metabolism and can indicate susceptibility to diseases. There is a wide range research projects running over the world which include metagenomic and genomic studies of microbiota to determine their composition, individual characteristics, and relationship to various factors such as age, diseases, gender, etc. One of the major factors in aging is regarded as the "regression of physiological functions" [19].

The decrease in the population and species diversity of many useful anaerobes, such as bacterioids and bifid bacteria, as well as changes in dominant representatives of the intestinal microflora, gives an insight into the reduction of functionality of microflora in the elderly.

One of the first to study changes in the intestinal microbiota was Dr. Mitsuoka. He observed that the levels of *Bifidobacterium* and *Clostridia* are reduced in old age, whereas the levels of lactobacilli, coliform bacteria, and enterococci are increased [20]. Many studies have been done since then to determine the peculiarities of the intestinal microflora in old age. The results depend also on the method of investigation. Typically, the decline in the level of a certain species was found only in works employing culture techniques which, probably, relates to changes in cultural characteristics of microorganisms. Hopkins and coworkers observed that the levels of *Bifidobacterium* and *Lactobacillus* were lower in the group of older patients compared to young respondents; at the same time, the levels of *Bacteroides*, *Enterococci*, *Enterobacteria,* and *Clostridia* were not different in both groups. A group of scientists guided by He [21] observed a high level of *Ruminococcus* and a lower one of *Eubacterium* and *Bacteroides*. Mueller et al. [22] investigated microbiota of elderly populations in France, Italy, Germany, and Sweden and found that age-related changes in the composition of microbiota were different in different countries. Regional changes in bifid bacteria and yeast cells were also observed in the adult population of Finland.

Age-related changes in the composition of intestinal microflora include increased numbers of facultative anaerobes and changes in the dominance of species while the total number of anaerobes remains stable [23, 24].

One of the common and significant age-related changes is reduction in the number of bifid bacteria [25]. While the composition of microbiota in an adult organism contains 4 to 5 species of *Bifidobacterium* genus, only one of the dominant species of this genus occur in old age: *Bifidobacteriumadolescentis*, or phenotypically congenial *Bifidobacteriumangulatum* and *Bifidobacteriumlongum* [26, 27]. One explanation of the decline in the species and quantitative composition of bifid bacteria in the elderly is the reduced adhesion due to changes in chemical composition and structure of the colonic mucosa [28, 29]. In turn, such changes result in limited functionality and immunologic responsiveness in the intestine as well as increased susceptibility to gastrointestinal infections.

Many authors show that diversity of *Bacteroides* species changes over time [30, 31]; at the same time, the presence of *Bacteroides thetaiotaomicron* is noted in all of the respondents [32].

Microorganisms of the *Bacteroides* genus are able to use a wide range of various carbon sources for their growth and activity, and they actively participate in the process of digestion of most polysaccharides in the colon [33, 34]. Changes in their abundance and diversity have significant implications for a macroorganism due to metabolic disorders and the presence of other microorganisms of microbiota in the complicated network of metabolic processes [35].

With age, concentration of *Clostridium* increases. *Clostrium difficile* is the most frequently occurring in old age, which may be due to nosocomial infection [36, 37]. He et al. observed a high concentration of *Ruminococcus* and *Enterobacteriaceae* cells in the microbiota of the elderly, along with low levels of *Eubacterium* and *Bacteroides* [29]. A team of scientists under the direction of Tongeren found that, in the microbiota of persons between the ages of 70 and 100, *Bacteroides*/*Prevotella*, *Eubacterium rectale*/*Clostridium coccoides,* and *Ruminococcus*

prevailed but the quantitative indicators were similar to the data by Harmsen et al., who studied the microbiota of healthy people aged 20 to 55 [38, 39]. In the intestinal microbiota of elderly people, growth of proteolytic bacteria such as *Fusobacteria*, *Propionibacteria,* and *Clostridia* was discovered [31, 40, 41], leading to the development of putrefactive processes, especially in patients after antibiotic therapy; this was confirmed by the data about increased proteolytic activity. *Clostridium* genus comprises a heterogeneous group of microorganisms with a wide variety of needs in nutrients and distributions. Ljungberg et al. [42] observed a significant decrease of *Clostridium* bacteria after introducing ciprofloxacin in groups of patients, both young and elderly. Unlike other scientific publications, the authors state that high levels of *Clostridium* in the intestinal microbiota of elderly patients are accompanied by increased species diversity: *Clostridium bifermentans*, *Clostridium clostridioforme*, *Clostridium sordellii,* and *Clostridium malenominatum* species occurring most frequently [31, 41, 43, 44]. In one case, a pathogenic representative of *C. difficile* was found, yet in another case, *Clostridium sporosphaeroides* [45].

The increased number of enterobacteria, streptococci, staphylococci, and yeast cells may be associated with an increase in serumal antibodies to commensal intestinal microflora such as *Escherichia coli* and *Enterococcus faecalis* [46].

In addition to changes in the microbial composition of microbiota, changes in microorganisms' activity are also observed. Due to the diversity of their enzymatic activity, the intestinal microbiota is capable of transforming various substrates entering the gut not only into body-beneficial metabolites but also into harmful ones. As a result of microbial metabolism in the colon, lactic acid, short-chain fatty acids, carbon dioxide, hydrogen, and water are formed. Carbon dioxide is largely converted into acetate; hydrogen is absorbed and excreted through the lungs; and organic acids are utilized by the macroorganism—and their value for humans is hard to be overestimated. One of the basic products of intestinal microorganisms are short-chain fatty acids (SCFAs) whose regulation was described in detail by Macfarlane [47] and Wong et al. [48]. Formed as a result of microbial metabolism, SCFAs are critical both for the colon and the macroorganism as a whole because they supply energy, regulate motility, maintain the stability of the intestinal microflora composition, and regulate apoptosis of epithelial cells of the colon [49, 50].

Decreased SCFAs and increased concentrations of branchiate fatty acids, ammonium, and phenols in an adult organism indicate changes in the activity of intestinal microbes, down to the development of adverse putrefactive processes [51]. Reduced production of intestinal SCFAs with age is partly associated with low consumption of dietary fiber [52] and antibiotic therapy [41]. Slow transit of masses through the gut affects the microorganism's activity due to the reduced availability of nutrients for a prolonged period of time, thereby contributing to the development of putrefaction. As a result of changes in the activity of microorganisms, pH factor of the intestinal lumen grows, solubility and absorption of minerals fall, and risk of infections increases. Moreover, the rise in concentrations of metabolites that are formed in the process of putrefying increases the risk of colon cancer [53].

Although lactic acid produced by bacteria such as lactobacilli and bifid bacteria is normally utilized by other types of microorganisms, it may, in the body of elderly people, be accumulated in the large bowel [54]. Studies by Tiihonen et al. demonstrate an increase in the concentration of lactic acid and bacteria producing it in the large intestines of the elderly, along with a reduced concentration of microorganisms that utilize lactic acid such as *C. coccoides* and *E. rectale* [55].

For the most part, these changes are pertinent to age-related characteristics and reflect only average features. However, due to lowered adaptive capacities in older age, any adverse effects lead to the formation of a pathological condition called dysbacteriosis.

Dysbacteriosis is most commonly characterized by a decreased total number of microbes, sometimes up to complete disappearance of certain types of normal microflora, with simultaneous predominance of species that are normally present in minimal amounts. Such predominance may be long-term or appear intermittently. In elderly patients, intestinal dysbacteriosis occurs

more frequently than in the young age, with most cases being severe disorders of microbiocenosis (dysbacteriosis of grade 2, 3) [56–58]. The scientific literature has limited information about the peculiarities of dysbacteriosis development in senior patients. Clinical manifestations of dysbacteriosis against the background of polymorbidity specific to older patients have not been specified so far [59–61].

5.3 POTENTIONAL BENEFITS OF PROBIOTICS, PREBIOTICS, AND SINBIOTICS FOR ELDERLY PEOPLE

Healing properties of lactic acid bacteria are well known. Their effectiveness is shown after antibiotic treatment and in correcting age-related disorders of the bowel microbiota in the range of researches [29, 44]. *Bifidobacterium* are very important—their numbers reach 10^{10} per gram of coprophiltrat dry weight [62].

After Mechnikov suggested using fermented milk in suppressing the putrefactive colon bacteria, it was noticed with increasing interest that there are possible health advantages in probiotic microorganisms [63, 64].

In 2002 the UN Food and Agricultural Organization defined probiotics as "living microorganisms, which give therapeutic effect for human organism in the case of its introduction in sufficient quantity" [65]. Numerous researchers discovered the possibility of probiotic use and emphasized their advantages, including prevention and treatment of some diseases such as inflammatory bowel diseases and lactose intolerance. The wide range of different probiotic food (milk and bakery products), medications, and dietary supplements a significant increase in the number of consumers consuming probiotics.

In 2008, the probiotic global market (including food and supplements) was more than $15.7 billions, with more than 70% coming from probiotic use in the United States, Western Europe, and Japan. Since 2003, the probiotic world market has doubled [66], and the specialists say it will reach $31.2 billion in 2014, and it will increase by 11.7%, at the average, in comparison to 2009 [67].

One of the primary reasons of the probiotic market growth rate is the increasing effectiveness of probiotic microorganisms and other components, which increase the biotherapeutic properties of the final product. One of the main benefits of probiotics relates to aging, because advanced age and improper feeding are important factors that are responsible for gut organisms and probiotics are essential in the solution to this problem. As well, it was noticed that there was an increase of consumer need to prevent the disorder of functions and stimulate the immune system [67].

The American Microbiology Academy had a colloquium in November 2005 and 38 specialists of various spheres attended. The questions about the interaction between microorganisms, immunity, and sicknesses; the evidential data in respect to probiotic treatment properties; and possible perspectives of their use were discussed [68, 69].

Careful study in experimental and clinical conditions showed certain probiotic effects, but the effectiveness and reproducibility of remedial measures with probiotics are yet to be confirmed.

Generally, probiotics are used as preventive remedies and concomitant treatment, but they are not a main treatment of sickness. The indications for probiotic use will be expanded in the future [70, 71], including these examples:

- Biotherapy using antibiotic-sensitive bacteria;
- Prevention of translocation of pathogenic bacteria from skin and mucous coats in the internal environment;
- Faster weight gain;
- Elimination of some types of bacteria from the human organism; for example, *Helicobacter pylori* in patients who have a gastric ulcer;

- Recovery of microflora composition after antibiotic treatment;
- Changes of bowels microflora composition in accord with individualities of the diet;
- Improvement of oxalate metabolism by way of reducing the frequency of calculi formation in the kidneys and urinary bladder;
- Breaking down potentially dangerous chemical substances, especially in cases of their permanent influence on the human organism; for example, at work;
- Reduction of the pathogenic *Staphylococcus aureus* and *C. difficile* from inpatients; and
- Prevention of urinary bladder infections.

Taking into account the potential danger related to using living microorganisms, including infection expansion, the perspective next step in probiotic use is to replace living microorganisms on individual components of microbial cells and products of bacterium exchange. Such an approach may be more effective. When the bioactive molecules that provide the actions of effective probiotics are found out, just these molecules can be used in their pure form. SCFA, peptidoglycan cell walls, and DNA are products of metabolism derived from bacteria that are components of probiotics, and can be used to direct treatment.

Currently, probiotics are not registered as biological medications used for the prevention or diagnosis of any human sickness in the United States. Probiotics are manufactured according to standards developed for food, but not in accordance to stricter standards for biological products [72]. Accessible probiotics are often characterized by uncertain quality. Either microorganisms will be not listed on the label or they will be in small quantity in the product.

Searching for a microorganism that can be used as a probiotic is a long and complex process. The first priority of probiotic composition should be safety. Microorganisms should not be pathogenic, special attention should be paid to the possibility of infection development in people with immune deficiencies. Plasmid-containing genes, which are responsible for antibiotic resistance in the culture of probiotics, should not be present as these genes can be passed to pathogenic bacteria in patients. The microorganisms should be resistant to acid and bilis to avoid reaching the supposed colonization zone. It is necessary to use in vitro and in vivo models for specification of probiotic mechanisms [65].

Carefully planned (randomized, placebo-controlled) clinical testing of probiotics should be undertaken, with wide investigation into patients' microflora, well-defined end points, and well-informed patients participating in the treatment.

Each presumed effect of a probiotic should be connected with a certain strain that is in its composition. The effects that are typical of a certain strain should not be ascribed to another strain of this species. The study of the mechanisms of each probiotic is necessary to find out the possibility of a general mechanism to explain their activities in different physiological and pathological conditions.

However, particularly in the United States, there is a fear of microbes and a belief that they should not be present in human organism. It is a false impression, and social educational programs should influence the misconception [65].

It is necessary that the bacteria react with each other in the human organism, and this reaction can play a determining role in supporting health. The probiotics' abilities to interfere in these reactions necessitates the studying of different types of normal microflora (including children, elderly people, and people with immune deficiencies) to ensure the safety of the appointed probiotics. It is necessary to understand the fundamental basis of a microorganism's ecology.

The search for new methods of treating childhood diarrhea appeared recently because of the increasing resistance of digestive pathogens to antibiotics. Methods of vaccinating against enteric infections, such as cholera and rotavirus infection, are developing. In addition, pharmaceutical companies are searching for new antimicrobial medications for diarrheal treatment [73].

Another method of diarrheal treatment is in the use of probiotics or nonpathogenic microorganisms colonizing the intestinal wall and limiting the overgrowth of pathogenic bacteria. The competition for the receptors of the mucous coat reduces adhesion and the growth of gram-negative enterotoxicant micronychia anaerobics and enteropathogen viruses allow for the reproduction of more "useful" bacteria on the mucosal surface.

The probiotics, such as lactic acid bacilli and bifid bacteria, secrete components with antibacterial properties and suppress the vital activity of digestive pathogens: the increased production of volatile fatty acids. The deacidification of digestive contents can also suppress the reproduction of the digestive pathogens. Some medications composed of probiotics, for example, *Lactobacillus* GG, have an immunomodulatory effect and can decrease the intensity of inflammation of the digestive wall.

At this time, several studies show the advantages of using probiotics in pediatric practice in hospitals as well as in ambulatory conditions. However, the completed studies had different designs and used different antibiotics which makes comparing the results difficult. Scientists from Children's Hospital Boston and Harvard University, in cooperation with scientists from New York University Medical School, carried out research to prove that probiotic use by children younger than 5 years old decreases the time of the diarrheal episode [74].

An article published in *Digestive Diseases and Sciences* showed the results of a meta-analysis study of the treatment of children with diarrhea with probiotics. Recently, many similar studies have been carried out, but most of them were uncontrolled and nonrandomized. Eighteen studies with adequate design (control and randomization) showed a reduced length of diarrheal episode during treatment with probiotics together with standard peroral rehydration [73]. In accordance with meta-analysis results carried out by researchers from Great Britain, probiotics (especially yeast fungus and lactic acid bacilli) help to prevent antibiotic-associated diarrhea.

Probiotics are medications with living microorganisms that help sustain microbial balance in a macroorganism. According to an article published in the *British Medical Journal*, pribiotics can be effective for digestive and vaginal infections. According to the researchers' opinion, there is nothing new in using living bacteria for antibiotic-associated diarrhea treatment [75]. But their use in clinical practice is limited. The use of living organisms gives a range of advantages and doctors should think about using these agents for the prevention of antibiotic-associated diarrhea.

Dr. D'Souza and his colleagues from Imperial College School of Medicine, London, carried out a search for randomized controlled studies published in the period from 1966 to 2000, using MEDLINE and The Cochrane Library, comparing probiotic and placebo use in patients [75].

In 2 out of 9 studies, the use of probiotics in children was studied. In 4 cases, yeast fungus (usual culture yeasts) was used, in another 4 cases lactic acid bacilli were used, and in 1 study strains of *Enterococcus*-produced milk acid were used. A combination of probiotic strains of bacteria was used in 3 studies. Probiotics and antibiotics were used together in all 9 cases and the control groups took antibiotics and placebos.

Scientists summarized the findings and discovered the advantages of probiotic therapy. The number of diarrheal cases caused by antibiotics was 63% less than in the control group, where taking yeast fungus decreased the frequency of diarrhea by 61% and lactic acid bacillus by 66%.

In addition, probiotics are becoming more accessible to people, and taking them is cheaper than taking antibiotics for treatment, such as for diarrhea. It is the author's opinion that taking probiotics can reduce the total time patients stay in the hospital. Studies prove that probiotics are effective for diarrhea prevention, but the number of such studies is not many. Further controlled clinical studies using different probiotics for prevention and treatment of this condition need to be conducted [76].

The spectrum of probiotic properties depends on metabolic characteristics, surface molecules, or secreted cell components. The essential components such as DNA or peptidoglycan also can have significance. The individual combination of such properties in certain probiotic strains

determines the specific mechanisms of probiotic action and, as result, its effectiveness in the prevention and/or treatment of sicknesses.

Kazakhstan has many potential probiotic products such as koumiss, shubat, ayran, kurt, and cheese. For a very long time koumiss was used as an antivenin. Koumiss therapy using for pneumonic tuberculosis, anaemia, saccharic urina ambrosia, adiposity, diseases of the nervous system, and inflammatory disease of the stomach and bowels. Koumiss is called the drink of longevity. Cultured milk shubat also has healing properties and there are specialized facilities that treat with shubat.

The therapeutic potential of these previously mentioned products in studies of probiotic base strain mechanisms is relevant and significant today.

Nowadays they are widely used for the prevention and treatment of antibiotic-associated diarrhea; nevertheless, the question about the possibility of these microorganisms surviving during simultaneous use of antimicrobial medication is still unanswered.

During the microbiological research that was done by M.R. D'Aimmo et al., the practicality of including bacterial probiotics in the composition of a range of commercial milk products and medications was studied [75]. Based on the outcome of the research, it can be supposed that simultaneous use of probiotics (especially lactic acid bacilli and bifid bacteria) with most common antibiotics is questionable, as probiotic microorganisms are sensitive to many antimicrobial medications.

Lactobacillus and bifid bacteria are representatives of normal human and warm-blooded animals' microflora, which are examined as a qualitative and quantitative ratio of microbial population of the individual organs and systems supporting the biochemical, metabolic, and immunological balance of host organisms [77].

The stimulatory influence of symbiotic lactoflora on the water-salt metabolism, glycometabolism, and the metabolism of proteins, lipids, nucleic acids, steroids, and other physiologically active compounds is known. Lactic acid bacilli have biological properties, including antagonistic, colonizing activity; acid formation; antibiotic resistance; and immunopotentiation, antineoplastic, antimutagenic, and cholesterin-utilized properties [78–86]. The inhibitory activity of lactic acid bacilli with opportunistic pathogens, pathogen microorganisms, yeasts, and viruses is related to the formation of antibiotic-similar substances (bacteriocins); production of milk acid, alcohol, lysozyme, and hydrogen dioxide; and the capability of adhesion [87]. Lactobacilli are resistant to lysozyme, digestive enzymes, acids, and bilis [88–92]. High adhesiveness to mucous coats and a weak antigenic load facilitate the development of a close association of mucous coats with the formation of the surface-protective biolayer.

It is known that lactic acid bacteria are suppliers of a range of essential amino acids and vitamins. Also, it was determined that they can neutralize the negative consequences of hereditary unsoundness in microorganisms' enzyme systems [93, 94].

Lactic acid bacteria are still one of the most commonly used probiotic microorganisms. *Lactobacillus* spp. often appears in food production for functional nutrition, together with bifid bacteria [95].

In recent years the interest in fermented products with probiotics has grown. It was found out from research results that some probiotic strains can change the activity of bowels' microflora by raising the bifid bacteria survival coefficient [96].

The number of studies showing immunomodulatory effects of probiotics [93, 94] is growing. The effects include stimulation of phagocytic activity of neutrophils, macrophages, immunoglobulin synthesis, and the formation of interferons, interleukins, and tumor necrosis factor (TNF) [97–99].

It is possible that probiotic bacteria and their metabolic products are absorbed with M-cells and replaced in deep-lying lymphatic follicles where they interact with immune competent cells [100], such as macrophages and T-cells. These interactions cause the production of cytokines, which cause different effects on immune and other cells [101].

While studying the influence of *Lactobacillus plantarum, Lactobacillus rhamnosus,* and *Lactobacillus paracasei* ssp. on the production of cytokines with peripheral blood mononuclear cells (PBMC), it was discovered that all researched bacteria increase the secretion of interleukin (IL)-12. Cytokines interact directly and indirectly with the human immune system. Interferons suppress virus replication, stimulate the expression of HLA I and II antigens, stimulate helper-inducer T-cells, activate macrophages, and enhance the potency of vaccines. IL-1 stimulates T- and B-cell proliferation. IL-6 cause the differentiation of B cells into plasma cells, and TNF-α has a cytotoxic effect on tumor cells [101].

The influence of probiotics on the humoral component of the immune system is being actively studied. Probiotics can increase animals' resistance to pathogen viruses. One study has shown that intergastral injection of *L. casei* and *L. acidophilus* in mice has been accompanied with an increase of plasma cell quantity and enhanced antibody synthesis to influenza viruses and rotaviruses [102–105], and also immunoglobulin (Ig)A and IgM synthesis increases on mucous coats of the bronchi [106].

The ability of probiotics to activate natural killer (NK) lymphocytes was discovered. At the time of incubation of lactic acid bacilli with human monocytic cells, the mobilization markers CD69 and CD25 were defined only on NK lymphocytes. Also it was proven that intranasal and inner injection of *L. casei* (strain Shirota) in mice stimulates functional NK lymphocyte activity in peripheral blood and respiratory organs [107]. Some studies [104] showed that the immunostimulatory effects of probiotics depend on the bacteria dose. The maximal level of Ig producers in the small bowel wall was observed during intergastral injection of bifid bacteria and lactic acid bacilli in the dose $5*10^{10}$ of microbial bodies.

It was shown that addition of *L. paracasei* (NCC2461) into the lymphocyte culture oppressed the proliferation of $CD4^+$ lymphocyte, which was accompanied with an increase in anti-inflammatory cytokines IL-10 and transforming growth factor β (TGF β).

The anticarcinogenic role of many probiotic strains is known today [108]. Antitumor activity is found in *L. casei* and *L. acidophilus* [109]. The basis for the anticarcinogenic action of lactic acid bacilli includes: the mechanisms of suspected carcinogen inactivation contained in food, the inhibition of the enzyme strength that is producing the mutagens, the immuno mechanism's stimulation of antitumor protection, and production of chemicals which can inhibit the tumor cells' proliferation. Most likely, it is caused by polysaccharose of lactic acid bacilli cell walls and polysaccharoses that are excreted into the environment.

A drop of promoters of tumor growth such as free amines, athoreductase, nitroreductase, and others can be observed in feces under the influence of probiotics. The drop of pH bowel secretion reduces the formation of secondary bile acid and the prevalence of promotion of cancer. Lactobacilli take part in the biotransformation of bile acids, steroid hormones, and oxalic acids in control of serum cholesterol rate and blood sugar.

Japanese investigator Hideki Ishekava, within the scope of clinical investigation, found the possibility of preventing re-differentiation of oncologic pathology using *Lactobacillus casei* strain Shirota. Findings of the investigation point out that the application of *Lactobacillus casei* Shirota prevents the development of colorectal cancer with moderate and hard atypia and recommends using *Lactobacillus* spp. Shirota in rectal cancer prevention.

Lactobacillus spp. anticancer activity was shown on Lewis lung carcinoma, melanoma, and hepatoma of linear mice.

Probiotics possess the highest anticancer activity in sarcoma. Compared with the control group, the prescription of probiotics in 58 bladder cancer patients within the year reduced the risk of recurrent tumor 1,8 times after providing the specific treatment.

There are data that suggest representatives of the normal microflora are able to attack the cholesterol, using it as energy source. Some of the *Lactobacillus* strains exert on cholesterin level in blood serum (e.g., strain GG *Lactobacillus* spp.). According to the clinical study of GG *Lactobacillus* spp., 35 volunteers who took cultured milk foods fermented by this strain had a drop of cholesterol levels within 2 weeks.

According to studies, the strain GG *L. casei* is able to stabilize the intestine's mucositic barrier, reduce the inflammatory process in the intestine of patients with atrophic dermatitis and food allergy, normalize immunological shifts, prevent the absorption of food antigens, and decrease the permeability of mucus of the intestine.

There are convincing data that some of the probiotic strains can reduce the risk of stone formation, as they possess oxalate modifying characteristics. It is proved that strains *L. acidophilus, L. plantarum, Lactobacillus spp., L. delbrueckii, L. fermentum,* and *L. helveticus* are able to reduce renal oxalate excretion.

It was found that probiotics affect blood pressure. Blood pressure reduction is noted in hypertensive users who take the *Lactobacillus* spp. They suspect that the substance responsible for this antihypertensive effect, which presents in an extracted lysate of cytoderm *L. casei,* is polysaccharide-glycopeptide complex, which was called as SG-I and its molecular weight is about 180,000. The prescription of SG-I of 1 to 10 mg/kg considerably reduced blood pressure. Probiotic bacteria possess a certain proteolytic activity, which is a condition of the effect of proteinase and peptidase. The proteolytic activity can be displayed by coccal forms, and by thermophilic *Bacillus* and *Streptobacillus*. During the milk protein proteolysis process, especially at the initiation of strain cultivation in milk, amino acid and peptide accumulate. Lactic acid rodlike bacteria possess more proteolytic activity than coccal forms. *L. delbrueckii* and *Lactobacillus* spp. can transfer 25% to 30% of casein into soluble form, whereas *Streptococcus cremoris* and *Streptococcus lactis* only transfer 15% to 17%.

Thereby, probiotic bacteria possess different biological properties, including: actively taking part in metabolic and regulatory processes of the macroorganism, being objects of studies for developing probiotic preparations, and being products of functional feeding for the correction of microecological disorders.

The results of analyses published in leading papers make it possible to define three main problems of advanced age: malnutrition, stool retention, and the reduced efficiency of the immune system—all of which can be avoided by taking probiotic products daily.

Aging doesn't affect the functions of gastrointestinal tract much, but due to the reduction of adaptive capacity in the gastrointestinal tract of elderly people, they cannot recover from diseases quickly. The reduction of adaptability also reduces tolerance to drugs. The reduction of the time it takes to empty the stomach produces expansion of satiation and higher risk of unbalanced feeding in elderly people.

The reduction of thirst threshold also has a negative influence on the defecation process and water balance. In connection with the reduction of taste sensation, elderly people usually consume less cellulose, or starch of carbohydrate, which reduces microbal fermentation in the large intestine. Small bowel bacterial overgrowth, reduced stomach acid secretion may lead to nutritive and vitamin malabsorption. Malnutrition is a risk factor for several diseases, such as osteoporosis and sarcopenia.

A general problem for most elderly people is the reduction of intestinal function. It may be the result of reduced physical activity, reduced fiber or water intake, or reduced intestinal motor activity—or may be any combination thereof. Probiotics are used for smooth of defecation process. Ouwehand et al. [128] observed that taking a combination of probiotics *L. rhamnosus* Lc-705 and *Propionbacterium freudenreichii* ssp. shermanii JS increased defecation frequency from 2.1/week to 2.6/week.

Studies demonstrating the application of probiotics and synbiotics as extra therapy in elderly patients are lacking. Placebo-control studies of elderly volunteers were carried out by a group of Dandy University scientists in 2005; these studies showed that application of probiotic medicine consisting of *B. lactis* BL-01 and *B. bifidum* BB-02 in conjunction with probiotic inulin leads to an increase in copra filtrates of bifidobacteriums and lactobacillus. In application, the detailed composition of specific variations of bifidobacteriums showed various types that are not in the composition of probiotics, particularly *B. adolescentis, B. angulatum,* and *B. dentium.* Although

those studies were carried out with a group of healthy people, the results show the potential of probiotics for people with major problems, especially those taking antibiotic therapy. Extra studies of microflora show seeding of bifidobacteriums within 3 weeks after discontinuation of probiotics. Results show not only the vicarious mechanism of corrective effect, but also the bifidogenic properties of medicine that stimulate growth of microflora, which probably connect with probiotic components.

The conception of prebiotics appeared more recently than probiotics; they represent chemical substances, usually oligosaccharides, that specificly act on and stimulate the growth of intestinal microflora. The choice of prebiotics is based on their nondigestibility by macroorganism and that they are metabolized by nonprobiotic representatives of intestine microflora, such as *Bacteroides* spp. and *Escherichia coli.* Prebiotics naturally come into an organism with breast milk, and they are also present in some vegetables (e.g., topinambour), synthetic oligosaccharides based on fructose and galactose also exist. Galactose can be used in the composition of functional food products or in concordance with probiotics. Fructooligosaccharide, galactooligosaccharide, inuline, lactulose are used as prebiotic substances in composition of probiotic medicines. Prebiotics are substrates for bifidobacteriums and lactobacteriums, which catabolyze them into molecules through osmotic effect, thus leading to eccoprotic effect.

Complex pre-/probiotic medicine is increasingly found as food supplements, which contain sublimate bacteriums in capsules, pills, and powder. The most popular probiotic product is yogurt, which is prepared by fermenting cow or goat's milk which changes lactose into lactic acid and adds probiotic microorganisms. As a rule, yogurt, which contain probiotic organisms, is called bioyogurt. Some probiotic products also contain prebiotics fructooligosaccharide and galactooligosaccharide. It is well known that the application of fermented milk products partly solves the problem of lactose intolerance. Because the low rate of β-galactosidases in mucous worsen with age, extended application of bioyogurts is a preventive measure for osteoporosis development.

Quantitive and qualitative changes of intestine microflora composition stimulate a drop in intestinal motility and, consequently, constipation occurs. This phenomenon is linked to a reduced concentration of bifidobacterium and in 1935 it was demonstrated that using lactic acid bacteria eases constipation. Investigators found improvement in the defecation processes of Japanese workers who eat yogurt with bifidobacteriums for an extended time. The group of scientists under the direction of Ouwehand [138] found that commercially available probiotics that contain *L. rhamnosus* LC-705 and *Propionibacterium freudenreichii* JS increased defication frequency in group of elderly people by 24%. It was also confirmed by Matsumoto, Salminen, and Umesaki [139–141].

As previously mentioned, aging reduces the function of the immune system, which can be observed by increasing infection processes, autoimmune diseases, and tumors that progressively affect the aging population. Studies showing the reduction of immune system function and correlating this process with age-related changes of the whole organism contributed to the formulation of the immunologic theory of aging. The first postulates of immunologic theory were offered by Bernet in 1950 to 1960. More recently, the theory was improved by Walford, Makinodan, Humphrey, White, Miller, Joffe, Anisimov, and Khavinson.

The ability of organisms to mount an effective reaction to disease decreases with age; this phenomenon is known as "immunosenescence," the aging of the immune system. Cellular immunity is decreased the most: the number of circulating CD3 lymphocytes is low and the activity of killer cells drops.

Various probiotic bacteriums, including yogurt organisms such as *L. johnsonii* La1, *L. acidophilus, L. casei*, and *B. lactis* Bb12, demonstrate immunostimulating properties in different in vitro and in vivo experiments, including modulating cytokine production, increasing phagocytic activity, adjuvant effect on specific humoral answer, and increasing function of T lymphocytes and activity of natural killer cells.

Gill and coauthors observed the positive effect of a 3-week course of *L. rhamnosus* HN001 in a group of elderly patients [142]. Also found were an increase of ά-interferon levels, total number of lymphocytes, and circulating cells $CD4^+$ and $CD25^+$. Improvement of immunological function in group of patients older than 70 was the most interesting result. Van der Water et al. [143] found a decrease in allergic diseases and levels of serum IgE in a group of elderly patients as a result of eating bioyogurt for a year.

In other study, carried out by group of scientists under the Guigoz's direction, it was shown that the consumption of prebiotic fructooligosaccharide for 3 weeks led to an increase in the number of bifidobacteriums, lymphocyte, and CD4 and CD8 cells. Reduction in phagocytic activity by polymorphonuclear cells and monocides, and a decreased interleukin-6 expression by monocides of peripheral blood were also observed.

Turchet and his colleagues [150] studied patients who ate bioyogurt with *L. casei* for 3 weeks and found that it led to decreased levels of "winter infections" (gastrointestinal and respiratory) in a group of elderly patients—20% fewer occurences when compared to the control group. Evidence of this link comes from data from clinical studies of elderly patients, which show consumption of probiotics increases the rates of immune system response. Some of the studies described an increase in phagocytic activity. Elderly people have a higher susceptibility to disease, especially after a prolonged infectious disease, which predisposes elderly patients to reinfection or a new infection. Consumption of *L. casei* probiotics within 3 weeks by 360 patients older than 60 with gastrointestine and respiratory infections showed a decrease in disease duration by 20%.

Latent infections play a significant role in aging. These slow processes, and also chronic inflammatory disease, lead to the early aging of an organism. Weakening of immune system function leads to increased risk of chronic diseases, such as autoimmune processes, atherosclerosis, pancreatic diabetes, and even Alzheimer disease.

Proinflammatory cytokine interacts with products of specific peptides connected with Alzheimer pathology, which means chronic inflammatory conditions of elderly people or circulation of inflammatory factors such as interleukin-6 and C-reactive protein, which contribute to Alzheimer disease. High concentrations of interleukin-6 and C-reactive proteins are also found in the blood of overweight people. These factors are associated with a risk of diabetes and high mortality. As some clinic studies show, taking probiotics and prebiotics reduce inflammatory biomarkers of elderly patients. In particular, the presence of specific types of bifidobacteriums in coprofiltrate can be associated with decreasing interleukin-10 serum, of tumor necrosis factor, or decreased expression of tumor necrosis factor or interleukin-6 in peripheral blood.

Various sections of the immune system can be attacked in different ways. Aging, is associated with a defeat of immune system cells; which is seen especially in the decrease in function of $CD4^+$ T-cells or contraction of CD8 cells by changing regulations exerted on the immune system. The fall in function of T-cells can worsen across the lifespan. The decrease in function of cells' immunity can improve antibody-associated effects in adults, as shown by studies linking probiotics with a reduction in the duration of winter infections.

Daily consumption of *L. rhamnosus* HN001 and *B. lactis* by patients older than 69 within 6 weeks of *Staphylococcus* infection demonstrated increased functional activity of phagocytal cells in the peripheral blood. Taking lower doses of probiotics for a shorter amount of time is sufficient for analogous effect. A study of 50 patients older than 60 after a 3-week course of probiotic *B. lactis* HN01 demonstrated the increase of phagocytes, which are stimulated by K562 tumor cells and mitogen, and in a study of 30 patient at 69 years of age, increased phagocytic activity induced *E. coli* K562 tumor cells. A combination of *B. lactis* HN019 and *L. rhamnosus* HN001 demonstrated increasing antitumorous activity of natural killer cells after consumption for 3 weeks in 27 patients at the age of 69.

A study of 51 patients older than 65 taking a combination of *L. acidophilus NCFM* and lactitol twice a day found improvement in the local immune status of the intestine, which displayed a

high concentration of prostaglandin E2, contributing to the growth of epithelial cells and their protective effect. It is especially important when taking nonsteroid anti-inflammatory medicine. Increasing the E2 prostaglandin concentration, which decreases with age, also contributes to improvement of intestinal motion function.

It is important that the presence of *B. longum* and *B. lactis* in coprofiltrate is linked to a lowered concentration of interleukin-10, and *B. lactis* with a lowered concentrate of tumor necrosis factor [160]. In a clinical study of 360 patients older than 60, patients reported frequent gastrointestinal tract pains, in flammatory respiratory diseases, and bronchopulmonary diseases. After daily consumption of probiotic *L. casei* DN-114 001, disease duration decreased by 20% (compared with the data of control group), but the incidence was not changed.

One aspect of taking probiotics is the prevention of cancer of the large intestine. Microflora of the large intestine contribute to the formation of colorectal cancer. The exemplars of intestine microflora are able to extract substances during metabolism which possess genetoxic carcinogenic activity, and are connected to disorders of microbe balance or product factors. This lead to studies that monitor food factors which beneficially modulate the intestinal microflora. According to studies, probiotics and prebiotics can modulate the metabolism of microflora. The survey of data (from animals, not people) shows the protective action of this factor in cancer of the large intestine, where strains of bacteriums that extract lactic acid and some of the prebitics prevent DNA damage from carcinogens in the large intestines of rats, but probiotics and prebiotics depressed precancerous overpatching in the large intestine of rats who took chemical carcinogens. In addition, there are arguments that synbiotics (the composition of prebiotics and probiotics) are more effective than prebiotics or probiotics when taken alone, and that mix of probiotics can be far more effective together than individually. In spite of data founded in experimental studies, the mechanisms of probitic, prebiotic, and synbiotic actions are not fully founded, but they can be used in chemical prevention of cancer of the large intestine.

We can define some of the most described mechanisms of probiotic effect. First, probiotics modulate the defense mechanisms of a microorganism, including inborn and acquired immunity. It is very important for the prevention and treatment of infectious diseases, and also for the treatment of inflammatory (chronic) diseases of the digestive tract. Second, probiotics can inhibit synanthropic and pathogenic microorganisms, which are very important for the prevention and treatment of infections and restore the microbial balance in the intestine. Finally, probiotic effects are based on the inactivation of toxins and detoxification of microorganisms. All three probiotic effects, in all likelihood, take part in protecting against infectious agents, preventing cancer, and stabilizing and restoring physiological equilibrium between intestinal microbiota and microorganisms. Likewise, we should stress that a single probiotic cannot display all three of these mechanisms together or, at least, an individual probiotic cannot be a prophylactic or treatment method for all types of diseases.

5.3.1 Production of Antibiotics and Similar Substances

Lactobacilli are characterized by the production of low-molecular bacteriocins, which include antimicrobial peptides. They are divided as follows: bacteriocins, including antibiotic posttranslational modified amino acids; bacteriocins, including disulfide bond of cystybiotic; bacteriocins with single SH remains of SH biotic; and bacteriocins without cysteine remains. In addition to bacteriocins probiotics are able to produce certain antibiotic substances. There are publications about production of reuterin by strain *L. reuteri* (3-hydroxypropionaldehyde). *Reuterin* is a wide-spectrum antibiotic, which is reactive to gram-positive and gram-negative bacteriums, fungal pathogens, and also viruses and protozoa. A specific antibiotic activity is a propery of proteins (molecular weight is more than 20 kDa), which belong to the bacteriocins family.

As the rule, such substances are produced by gram-positive bacteriums, but they also can be produced by gram-negative. Production of bacteriocins that possess reactivity to gram-positive and gram-negative bacteriums has been proven. In addition, probiotic bacteria are able to produce deconjugative fatty acids, which are derivatives of bile salts. Deconjugative fatty acids demonstrate stronger antimicrobial activity than bile salts, which are produced by macroorganisms.

5.3.2 Competition for Scarce Resources

An important example of a limited substance is iron; for almost all bacteriums, except *Lactobacillus*, iron is a necessary element. *Lactobacillus* do not need any iron in their life activity, which may be a decisive advantage when in competition with other microorganisms that depend on current nutrients. Studies have shown that, *L. acidophilus* and *L. delbrueckii* are able to connect ferric hydroxide on their own cell surfaces, which then makes it unavailable for pathogenic germs. As opposed to *Lactobacillus*, probiotic strain *E. coli N* depends on iron and hides siderophore chelates of iron or iron protoxide and absorps iron for transfer to bacterial cells. This property is similar for all pathogenic bacteriums, but *E. coli N* is able to compete very effectively, as long as it encodes at least 7 various systems of iron absorption.

5.3.3 Antiadhesive Properties

Probiotic bacteriums are able to adhere to epithelial cells, thereby blocking adhesion of pathogens. This mechanism is very important to the effectiveness of probiotics in a macroorganism.

An antiadhesive effect can be the result of a probiotic and pathogen competing for the same receptors or production of mucous by the probiotics. Studies by Mack et al. (2003) confirmed that the induction of mucin in HT20-MTX cells with *L. plantarum* 299v or *L. rhamnosus* GG can really be observed. In turn, mucin blocks adhesion of the enteropathogenic strain *E. coli* strain E2348/69. The study demonstrated that even adhesion of pathogenic strain *Salmonella, Clostridium,* and *E. coli* to the intestinal mucus of pigs decreased in the presence of probiotic strain *B. lactis* Bb12 and/or *L. rhamnosus* LGG. The pathogens' abilities to block adhesion to NH layer of the intestine most probably depends on specific probiotic strains as agents. In addition, some commercial probiotic strains even increase adhesion of *E. coli, L. monocytogenes,* and *S. typhimurium* to NH. Competitive exception (i.e., competition for the same receptors) by probiotics and pathogens, as said above, and other models of antiadhesive activity of probiotics can degrade carbohydrate receptors by secreted proteins, creating biofilm, producing homologous receptors, and inducing biosurfactants.

5.3.4 Anti-invasive Effect

Not only adhesion, but invasion of the epithelial cells is also a main property of bowel pathogenic organisms. Probiotics exist that are able to specifically block the invasion of bacteria. There are publications about inhibiting the invasion of probiotic strain *EcN* in bowel epithelial cells by pathogens such as *S. typhimurium, Yersinia enterocolitica, Shigella flexneri, Legionella pneumophila*, and *L. monocytogenes*. Secreted factors of some probiotic lactobacilli and strain *B. bifidum* Bb12 also block the invasion *S. typhimurium* into epithelial cells. The ability of epithelial cells to inhibit bacterium invasions of the intestine by pathogens is widespread among probiotics.

5.3.5 Antitoxic Effect

Probably the most important group of bacteria factors are toxins. For cases of diarrhea, efficiency of some probiotics is based on their antitoxicological abilities. This property may be the result of inhibiting the expression of pathogenic toxins. For strains *B. breve Yakult* and *B. pseudocatenulatum* DSM20439, in vitro and in vivo experiments demonstrate an inhibition of the expression of Shiga toxins in strain *E. coli (STEC)* O157:H7 versus other isolation bifidobacteriums. All animals who were treated by strain *B. breve Yakult* survived 90% longer than the control group of animals, who died after injection by strain *E. coli (STEC)* O157:H7. Through in vitro research, it is predicted that high concentrations of acetic acid, which are produced by the strain *Yakult,* inhibit Shiga toxin expression. Similarly, probiotic strain *Clostridium butyricum* MIYAIRI demonstrated activity against enteropathogenic *E. coli (EHEC)* O157:H7 in gnotobiont mice. Strains MIYAIRI inhibits, in vitro and in vivo, not only the expression of Shiga toxin by production of oil and milk acids but also reduces the viability of oil acid in strain EHEC through neutralization (pH 7). Real-time polymerase chain reaction (PCR) confirmed that 15 various probiotic strains of *Lactobacillus* inhibit expression of Shiga toxin 2A by producing organic acids on strain EHEC O157:H7 [186]. Some probiotics even display activity against toxins of fungus and cyanobacteria. The main observed protective action is probably the physical-chemical interaction between toxins and probiotics, rather than metabolic inactivation. Micotoxin deoxynivalenol, which contaminates grain crops, can lead to the development of gastroenteritis. *Lactobacillus rhamnosus* GG as a live culture, as in inactivated condition, is able to adhere to deoxynivalenol thereby limiting bioavailability of this toxin. *L. rhamnosus* GG and *L. rhamnosus* LC-705 are able to adhere to other micotoxins, and also aflatoxin. Studies of laboratory rats found that *L. rhamnosus* GG is able to modulate the absorption and increased expression of aflatoxins and, consequently, toxicity drops. This is because of the connection between aflotoxin and the probiotic.

Finally, not limited to strain *L. rhamnosus*, strains *B. lactis* and *B. longum* connected peptides of such cyanobacteria toxins as microcystin LR. This toxin is the most rapid and toxic and can be found in drinking water after contamination by cyanobacteria.

It is known that probiotics possess anticancer action, but what is the mechanism of this action? This question has not been well studied. At the very least, depression of putrefactive bacterium, such as *Clostridium*, colon bacillus, and other various bacteriums, and the resulting increase of the number of *Lactobacillus* and *Bifidobacteriums* can reduce the probability of colorectal cancer disease. There are cases about the decrease in adenocarcinomas of the large intestine in experimental animals after taking *L. salivarius.* Probiotics block chronic inflammatory disease of the intestine and, in doing so, decrease the risk of developing intestinal carcinoma, because chronic inflammation leads to its development. An example of the anti-inflammatory action of probiotics is the strain *Streptococcus thermophilus* TH-4 that produces huge quantities of folic acid, which is important for repair of DNA in epithelial cells. Antimutagenic activity was demonstrated for many lactobacilli strains, some bifidobacteriums, and EcN strains in an in vitro study. Properties of this probiotic include metabolic inhibition of mutagen substances, which is probably responsible for this activity. In addition, it was demonstrated that specific probiotics can connect N-nitrose molecules and hetero polycyclic aromatic amines. It may lead to a decrease in carcinogenic levels and less damage to DNA. One of the methods of anticancer activity of probiotics is their ability to increase immunity response at the expense of modulating cytokine production and the function of T-cells. The reduction of cancer proliferation in cells in vitro and increasing the survival of mice who were injected with tumor cells are both based on the modulation of cells' immunity response to the cytoplazmatic fraction of *L. acidophilus* SNUL*, L. casei* YIT9092, and *B. longum* HY8001. Another anticancer property of a cell's fraction is study of the peptidoglycan *Lactobacillus.* In a study of peptidoglycan, it cut the growth of tumorous cells CT26 derived from a BALB/C mice's intestine in a dose-dependent way. *L.*

reuteri ATCCPTA6475 produces factors that potentiate apoptosis in cells of MyLV, which is induced by tumor necrosis factor. This includes inhibition of IkBa ubiquitination and increases pro-apoptotic MARK signalling.

The influence of probiotics on functions of the immune system is observed in healthy patients as in patients with various diseases. Many authors point out a decrease of IgA secretory concentration in the presence of intestinal dysbacteriosis, and also taking probiotics containging *B. bifidum* and *L. Acidophilus* within 28 days restores the concentration of IgA. Besides establishing that, probiotics increase the quantity of T lymphocytes, T helpers, activated T lymphocytes ($CD25^+$) and natural killer cells in peripheral blood. Also, probiotics increase the phagocytic activity of neutrophils and monoacids of blood and the tumoricidal activity of natural killer cells.

The microbiocenosis is controlled by the immune system within the mucous coat of the intestine, and products of normal microflora, in turn, regulate cells' components of immunity.

Apparently, these immunomodulation actions can occur even in cases of absent live probiotic microbe cells, but the modulators are proteases, peptidoglycans, and also exocellular products. According to many works, muropeptide, peptidoglycan's component within probiotic cell walls, induces attack on macrophage, which increases the production of IL-1β, IL-6, and tumor necrosis factor. Rachmilewitzetal demonstrated a system of DNA anti-inflammatory effects by probiotic microorganisms, in order to show flammatory reactions by pathogen bacteriums of DNA. The mechanism of DNA effect is still unclear.

The adhesive reaction that probiotics have with the epithelial cells of a host organism causes the cascade of signals that leads to immunomodulation. Also, the freeing of soluble factor can cause the cascade of signals of immune cells, directly or by force of epithelial cells. When using live probiotic cells there are several mechanisms of interaction. First of all, such bacteria adhere on the mucous surface or connect with M-cells, which leads to stimulation of lymphoid tissue, cell or humoral immune response, and activation of cytokine production.

Interaction of probiotic cells with the mucous coat of the intestines stimulaes proliferation of immunoglobin-syntesing cells. B-lymphocytes that are induced in Peyer's plaques migrate into secretary organs, a condition of the immune system's development. This process connected with ability of LR to stimulate the differentiation endoglobular of T-cells, towards suppressor and cytoxical phenotypes, which causes cell-mediated immune reactions.

Interaction of the probiotic cells with the lymphoid tissue of the intestine leads to expression genes of proinflammatory cytokine IL-6, IL-1 β2, IL-1 β8, and tumor necrosis factor. IL-1 β, in its order to fall the synthesis of cytokine Th1 cells, inhibit the production by macrophage IL-1 β 2 and by tumor necrosis factor, decreasing the development of a cell-mediate immune response.

In studies where the strain *L. plantarum* was used, it was demonstrated that the TLR-2 dependent mechanism of protection developed from the amplification and redistribution of zonula occludens 2 (ZO-2) protein in cells T84 [211].

Inductions by probiotic strain *E. coli* Nissle 1917 (ECN) of ZO-1 and ZO-2's expression were demonstrated in experiments on laboratory rats in vivo. The Ukena et al. study demonstrated the anti-inflamnatory effect *of E. coli* Nissle 1917 on the model of colitis, induced by salt cake dextran, which caused weight loss and impaired function of large intestine.

Studies of *L. rhamnosus* GG found that the proteins strengthen the epithelial barrier, blocking TNF-mediated apoptosis by way of activation anti-apoptotic factors and protein kinase B. Besides, they inactivated miogen p38 in epithelial cells.

Finally, probiotics, as shown in in vitro, induce the expression of defencines and cryptidins in Panetta's cells. This mechanism possesses the main method of supporting a patient who has colitis in a period of remission from peptic ulcer diseases.

Studies have shown the subcutaneous introduction of probiotics can cause an anti-inflammatory effect, as seen in mice with colitis and also in treatment of arthritis. It explains by induction of probiotics of some regulative T-cells. A placebo-controlled study showed the two-way interaction of *L. casei Shirota* natural killer cells, by inducing production of IL-12. All the provided

examples show that probiotics attack the immunocorrecting effect, although this is only the first step to understanding the molecular basis of observed effects.

Analyses of various studies show that probiotics activate not only the local immunity of the intestine, but also the immune system of a whole organism.

Probiotics have multiple and polyfunctional influences on immune reactions: high-grade stimulation of nonspecific immunity factors, synthesis of mediators of cell-mediated immune reactions, and activation of the humoral component of immune response.

Ideal probiotic medicine should be given in these circumstances: first of all, microorganisms should be bile-tolerant, saline acid-tolerant, and pancreatic juice-tolerant; should produce lactic acid; and should be able to survive in the acidic medium of stomach and alkaline medium of duodenum.

In total, analyses of the literature allows to conclude the instability of the symbiotic stage of microbiota and the person's organism which are specific and typical characteristics in the process of aging.

Consequently, the "microbiota-macroorganism" system can lose symbiosis during aging and through dysbacteriosis it can turn into antagonism. Attempts to correct the interactions of the microorganisms and microbiota of the intestine lead to the use of probiotics, prebiotics, and synbiotics although the effectiveness is small. Using probiotics with a number of *Lactobacillus* and *Bifidobacteriums* is carried out without analyses of enterotype of intestine's microflora and describing the stage of microbiota. For objectification of selection of corrective interventions, specific biomarkers are obviously needed, which reflect the condition of microbiota and/or intensity of macroorganism's response.

Based on monitoring biomarkers, it's possible to pick an optimal combination of "correcting microflora" and probiotics to recreate the symbiotic stage of microbiota and microorganisms in the process of aging and, possibly in the future, the inhibition of the aging process and anti-aging treatment.

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Chapter VI

Development of Healthy Aging Innovative Programs: Gerontoengineering and Gerontotechnologies

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6.1 PRACTICAL VIEW OF DIFFERENT TYPES OF HUMAN AGING

Analysis of world literature and observations indicates that new approaches are needed in gerontology and healthy aging. The worldwide and steady increase in the elderly population is a challenge to humanity. The way the scientific community meets this challenge will, to a considerable degree, determine the destiny of all people and, undoubtedly, the development of civilization as a whole. The effective use of elderly people's potential and experience is an essential goal of every highly developed state. The development of government programs of science-based gerontology is a first priority and shall stimulate the development of social events aimed at improving life quality in issues of health and social security. In terms of scientific research, it is urgent to develop integrative biomarkers of aging, identify specific targets of interventions, and find probable regulatory interventions in the processes that result in pathologies, acceleration of aging or complications, as well as raising the quality of healthy aging.

Methodologically, it seems important to conventionally divide human aging into several categories: (a) normal or natural aging; (b) premature aging; (c) pathologic aging; and (d) healthy aging. Moreover, it should be noted that, considering present trends of societal development and the total increase of average life expectancy, age groups can be categorized as follows: "young-old," which includes people at the ages 65–74; "middle-old," 75–84 years old; and "oldest-old"

over 85 [1]. However, despite the possibility of such classification, chronological age does not always reflect biological predictors. According to Aubrey de Grey's theory, one of 7 types of damages in aging is loss of cells in various organism tissues [2] and, as a result, elderly people become less able to respond to various stress factors (or so it was thought). However, NIA GRC's research has shown that the general condition of some 80-year-old research participants is much better than that of younger subjects. For instance, organ function changes with age, which is why it is necessary to develop a system of biological age biomarkers because chronological age has proven to not be reliable for measuring of the productivity of an organism [3].

To date there have been many attempts to separate the process of normal (*natural*) from *pathologic aging*. The most significant, from scientific point of view, is the research conducted at the National Institute on Aging's Gerontology Research Center (NIA GRC) in Baltimore, Maryland. For 50 years this institute has studied key biological processes and social factors that affect normal, or natural, aging. This research (Baltimore Longitudinal Study of Aging, or BLSA) was necessary to lay the foundation for an effective model for improving quality of life and maybe its prolongation. For this research, a longitudinal design was chosen because it tracks the biological profile of each individual in a specific age group. Because information gathering from the research participants took such a long time, the subjects changed from one age group to another [4].

Today, BLSA's research has resulted in a number of important discoveries regarding the processes of *normal* aging, such as: tolerance to glucose, blood cholesterol level, alcohol metabolism, size of the heart and its function, memory, and many others. For example, when testing glucose tolerance, the normal level was considered to be the glucose level of a healthy 20-year-old subject whereas NIA GRC's research has shown that this standard is not appropriate for elderly people. Therefore, when this standard was applied to the elderly, more than half of them were considered to be diabetic. That is why the center's workers introduced recommendations and corresponding amendments for diagnosing diabetes [5]. Similarly, when testing kidney function, there were no standards developed for elderly and old patients. With new data obtained in the course of NIA GRC's research, corresponding standards and recommendations for diagnosing and treating kidney disease, considering age-related features, have been developed [6]. Thus, understanding of the *normal processes* of aging enables practicing doctors to achieve better results and avoid overdiagnosis of elderly and old patients.

Research of elderly patients' blood cholesterol levels is very interesting. An increase in cholesterol level is observed before the age of 55, and then a decrease takes place, thus dynamics significantly differ with age. Nevertheless, is should be noted that there are significant parameters that impact blood cholesterol level such as body weight, diet, physical activity, etc. [7, 8]. Other interesting research of NIA GRC scientists is study of age and alcohol metabolism. It has been established that elderly people's alcohol metabolism does not differ from young people, although equal levels of blood alcohol slow the memory and reaction of elderly people [9].

Promising results can be obtained regarding hormonal receptors and their significance in the process of *"normal"* aging. It is known that the number of such receptors reduce with age and tissue loss can be observed and prevention with the help of medicine is being studied. It is suggested that, in the future, mechanisms of cognitive and other physiological differences between people in different age periods will be discovered, despite the fact that process of individual aging is subject to the influence of social, economic, and behavioral factors.

In terms of understanding of aging mechanisms, study of *progeria* or *premature aging* is very important. Two main kinds of progeria course can be singled out: children's progeria (Hutchinson-Gilford syndrome) and adults' progeria (Werner syndrome). Both are characterized with accelerated development of common signs of *natural aging*, but in the first case they start developing from birth and patients seldom live to 20. In the second case, accelerated aging starts in adulthood, and the life span may be up to 30 to 40 years [10]. It is noted that people die from functional failures typical of extreme old age or from typical age-specific pathology, including cancer, heart failure, cerebral abnormalities, and other diseases.

It should be noted that an important observation of the BLSA's research was establishing that most measured factors change with age gradually, not by leaps and bounds. This observation allows for the suggestion that such abrupt changes more typical of development are associated with age pathology that is referred to as *pathologic aging*. Literature often refers to age-associated diseases, which are diseases that become considerably frequent with aging. They should be differentiated from diseases typical for a specific age group, such as some infectious diseases of childhood. We see age-associated diseases as diseases related directly to pathologic aging, including: cardiovascular system diseases, oncological diseases, diabetes type II, Alzheimer's disease, Parkinson's disease, etc. The mortality rate has dropped in the 20th century, especially in developed countries, because of better economic and social conditions, the introduction and development of sanitary hygienic measures, change of food, etc. Mortality evolution is significantly influenced by the very relation between pathologic aging and its associated diseases. Thus, deep insight into the mechanisms of age-related pathology can make a considerable contribution in determining the mechanisms of aging biology.

Healthy or healthier aging. When evaluating the aging of a population, it can be noted that examples of natural or normal aging are almost isolated and do not make a considerable part in the general cohort of the aging, and that premature aging is an extremely rare phenomenon. In the population, aging is developed according to disturbed or pathologic aging and is accompanied by age-associated diseases. It is desirable that researchers and society form a single understanding of the fact that aging is not pathologic and does not result on its own in so-called "age-associated" diseases, this is confirmed by observing groups of people with normal, natural aging. External manifestations of normal aging considerably vary between people. Focusing on "healthy" or "healthier" aging, we try to underline the main goal of a planned program of intervening in aging processes: transforming disturbed or pathologic aging into a course of aging without age-associated diseases and with an improved quality of health. We see this transformation as an active process of interference with the key links of aging and antiaging. It is also extremely important to mention that the very interference into the aging process at the level of human physiology and biochemistry is only a part of a larger program to achieve longer, higher quality life. By singling out the notion of healthy aging we try to represent the influences of complex measures (pharmacotherapeutic, medical, social) aimed at developing and maintaining qualitative macrobiosis.

We suggest that age-related changes of an organsim are an adaptive process of development and shows balance of aging itself and antiaging. This interaction also implies another key link: hormesis.

6.2 HORMESIS—AGING—ANTIAGING

A generally accepted definition of hormesis is "a process where influence of small doses of a chemical agent or environmental aspects causes adaptive effects and has a favorable impact on cells and on the organism as a whole, and the influence of large doses is destructive." It should be also noted that hormesis is an inherent part of the normal physiology of cells and organisms. Hormesis is also present as a main notion in evolution theory. Hormesis can be also regarded as a mechanism responsible for the protection of an organism's health against the impact of various lifestyle and environmental factors. There is a prevailing opinion that hormesis is a biological adaptive mechanism involving the participation of various tissues, organs, and systems of an organism [11]. Hormesis can be considered both an intermediary of external factors' influence on processes of health and aging and as a mechanism of regulation and coordination of adaptive mechanisms.

The idea to use hormesis for retarding aging and improving the health of the elderly population has been discussed in scientific literature [12]. Almost all researchers mention that aging is related to a decrease of the adaptive capability of an organism and a higher vulnerability to stress. However,

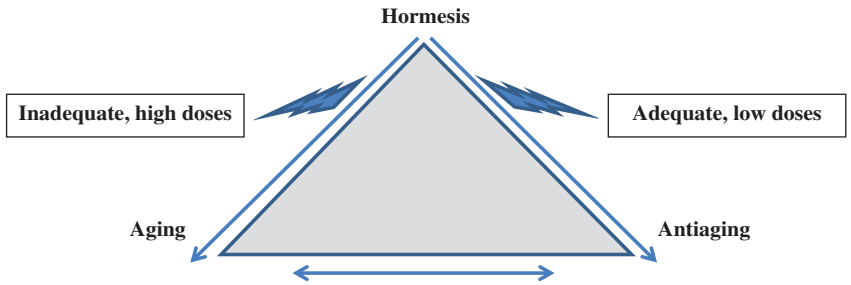

Figure 6–1. Possible influence of hormesis on aging and antiaging processes

on the other hand, it is clear that aging is a more complicated multifactoral process that may include activation of some stress-reacting systems which may be considered "geroprotective" [13]. In recent years the term "hormetins" has been used to indicate factors or substances causing hormesis [14] and able to stimulate independent cellular regeneration, increase adaptive capability and as a result slow aging. It is quite reasonable to search among hormetins for antiaging interventions and potential treatments of chronic diseases associated to age, including metabolic and cardiovascular diseases, cancer, and neurodegenerative illnesses [15].

So far, no data proves hormesis as an effective antiaging strategy which can delay aging and aging-specific human diseases, but this strategy works successfully in a number of model organisms (yeasts, worms, flies, rodents) [16–18]. Dangers that hide in the use of high doses of any "hormetin" are obvious and remain a major obstacle for practical realization of hormesis to be used for healthy people. The influence of hormesis on integral homeostasis of an organism and its systems is temporary or reversible and requires repeated exposures to achieve a stable effect. It is obvious that indicators of levels and peculiarities of hormesis influence are required.

Schematically, the influence of hormesis can be illustrated in the form of a triangle where the peak is hormesis phenomenon, and on the base of the triangle are processes of aging and antiaging (Figure 6–1). It is the imbalance of these "aging–antiaging" processes under the influence of hormesis (external hormetic impacts) that leads to various pathologic conditions and, eventually, with various diseases (Alzheimer's disease, Parkinson's disease; atherosclerosis, diabetes, malignant tumors), which are not necessarily programmed in all individuals. It is possible to classify hormetic influence on an organism into *adequate* and *inadequate*, depending on specific response. Available data make it possible to say that exposure is adequate when it's a low-dosage, variable or short application of a factor (external, physical, chemical, biological) to a living organism that causes weak or soft stress response reaction, which in turn activates adaptive reparative mechanisms resisting apoptosis and finally inhibiting aging processes of cells and organism. Inadequate, high-dosage exposure to the same factor that caused stress reactions, will accelerate the aging processes or damage antiaging mechanisms.

An example can be given using the most common agent used in gerontology resveratrol—as hormetic impact. From the results of research given in Chapter 5, a diagram was developed with possible options of an organism's hormetic response to application of various dosages of resveratrol geroprotector (Figure 6–2).

It is generally known that there is inconsistency in the results of application of almost all pharmacologic substances studied as geroprotectors; this scheme may serve as an explanation of the situation. At least, it is necessary to introduce a key link into the scheme; namely, characteristics and type of stress signal and intensity level of reaction of hormetic effectors. Qualitative or quantitative characteristics of stress cell reaction can be a criterion of adequacy

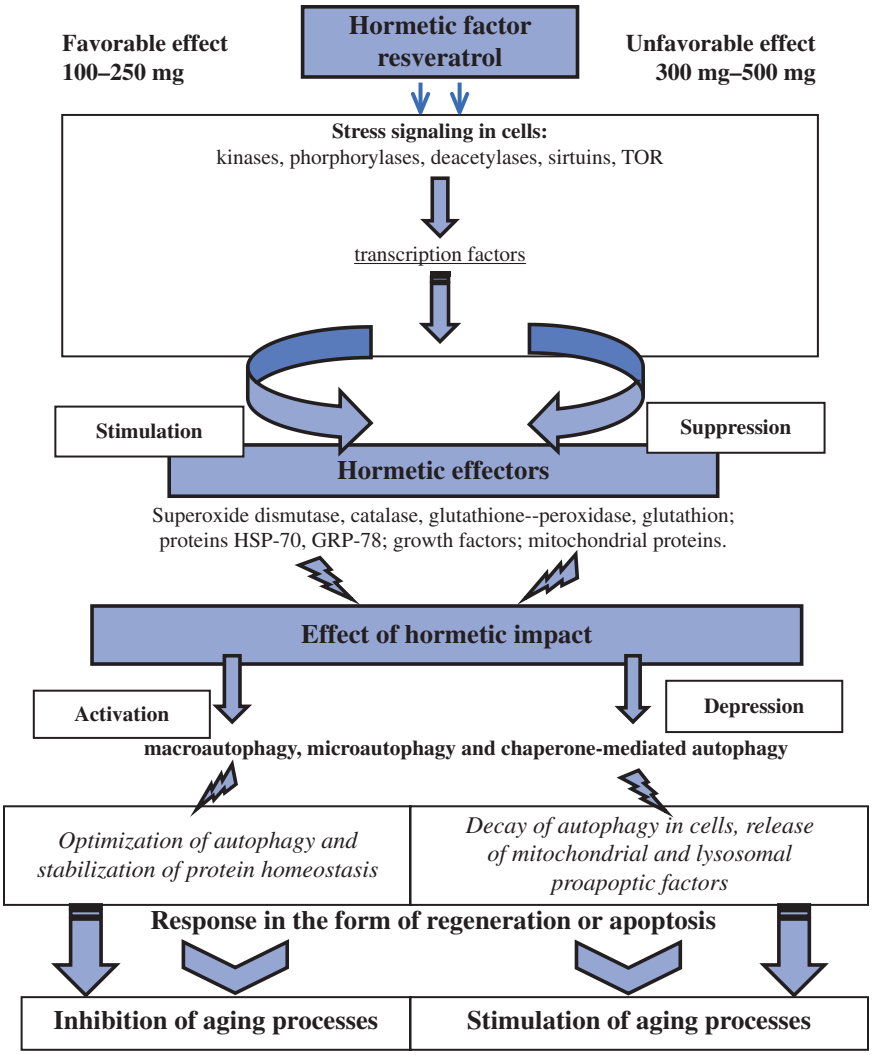

Figure 6–2. Hypothetical hormetic effect of resveratrol

of hormetic dose. Intensity of autophagy as a showing of hormetic impact also needs qualitative characteristic. Special interest is presented in the study of autophagy completion. There are a number of reasons to think that it is the showing that claims to role of cardinal and key one among markers of hormesis and biomarkers of aging.

In general, we definitely need means to measure the level of cellular and tissue stress when applying hormesis. The latter, most probably, may become complicated due to differences in the response of various tissues or organs, even within the same organism. Nevertheless, determining indicators or biomarkers of hormesis levels seems to be a promising direction of future research.

From our point of view, the notion of antiaging medicine or gerontology should include the prediction of external hormetic impacts and prevention of hormesis disorders which can result in diseases, and also the development of recommendations for improving health and ensuring quality long life. It is interesting and important to study and develop integrative biomarkers of age-related disorders of hormesis, which can result in pathologic conditions

and disturbed aging, and also system targets of regulatory influence on detected disorders with the purpose of their timely elimination. The search for and determination of aging biomarkers, in spite of great number of continuing research, has made a big contribution into understanding and developing many issues of gerontology development. At the same time, well-known problems of qualitative longevity development demonstrate the certain scientific and practical importance of the search for integral biomarkers of hormesis disorders, corresponding to different stages of man's life that is of big importance for understanding normal, premature, disturbed and healthy aging, and also will make contribution into development of integral system targets of influence, trigger points of regulatory interference and stable development of qualitative longevity.

The search for the means to prevent premature aging and the development of age-associated diseases is one of the priorities of the World Plan of Action and Aging Research Program in XXI century, adopted in 2002 at the Second World Assembly on Aging in Madrid [19]. It is generally known that the issue of aging was and remains one of the most topical in natural science. There have been discussions regarding the reality of success, timeliness, and reasonability of efforts and spending on this area of science.

Approaches to the practical realization of the processes of aging, antiaging, and rejuvenation so far have not resulted in increasing maximal longevity. Better living conditions, medical, and hygiene measures have only made it possible to increase life expectancy—to reduce death rates due to accidents, pathologies, and infections. The life-prolonging effect of "universal" intervention into the aging process by limiting calories is not developed enough for humans. Also, it has not been possible to develop effective and universal biomarkers of aging. Besides, at the moment, geroprotectors have a contradictory impact on longevity. Often their impact lies within the hormetic range set for nonspecific compensatory stress response (20–30%). That questions their target impact on aging mechanisms.

Thus, the previously existing methodology of interference into the processes of aging and antiaging is obviously insufficient for realizing qualitative longevity and rejuvenation.

At the same time, information continues to accumulate about the role of certain genes in regulating longevity in animal model and about reproducible change of expression of thousands of genes by various tissues' aging in connection with epigenetic changes. Fundamental success has been achieved in regenerative medicine, including reprogramming differentiated cells into stem cells and identifing the differentiation of controlling cells, or cytokines.

New knowledge has led to an understanding of the necessity to develop other gerontological approaches and new methodologies of interference in order to correct the processes of aging, antiaging, and rejuvenation. There has been an agreement achieved of almost all researchers of gerontology and geriatrics issues that, in addition to the aging process, there is also an antiaging process. At the same time many terms require more precise definition and understanding. It should be also noted that processes understood as antiaging have a general biological nature and are found at different stages of the whole ontogenesis—it is only in old age that they acquire some special features due to known reasons that witness conditional character of this term.

It has become possible to detect the manifestation of antiaging at different levels of organism. The antiaging mechanism is partly inherited. For instance, system of DNA reparation; antioxidant system protecting proteins, nucleic acids, cell membranes, etc. Other antiaging mechanisms are activated in the course of aging due to self-regulation (the activation of a number of ferments, a higher sensitivity of a number of cells to hormones due to weaker functioning of some glands, hypertrophy, and multinuclear character of cells, etc.). Some antiaging mechanisms are directed at creating systems that are stronger, more reliable, and age slower; the others are directed at compensation and liquidation of aging consequences. The balance of aging and antiaging processes determines the longevity of an organism. At the same time, many antiaging processes, represent compensatory adaptive reactions of the developing organism. It seems that the aging

and antiaging issue is immense. But we think it is possible to single out the most pragmatic and rather narrow field: developing an innovative program of qualitative aging and controlling the antiaging processes. It can be expected that within this approach, efforts will be focused on the following:

- Screening and testing of biomarkers of aging, antiaging, and hormesis;
- Determining correlation in biomarker systems;
- Detecting key points for interference in the aging processes;
- Selecting interference methods of aging, estimating inhibition methods of aging, and stimulating antiaging processes.

This book attempts to conduct an analysis of existing methodologies for aging, antiaging, and rejuvenation in order to determine the possible structure of a program for interference in key points of these processes and correcting their course. A critical analysis of existing approaches, ideas, and suggestions should be conducted to form a concept for development of scientific approaches to settle the problems of qualitative longevity and antiaging.

6.3 CRITICAL REVIEW OF THEORETICAL BASICS OF ANTIAGING TECHNOLOGIES

Antiaging medicine is a relatively new medical domain that has developed at a fast rate. It applies advanced scientific and medical technology for the prevention, early detection, treatment, and cure of age-related disfunctions, disorders, and diseases.

The aim of antiaging medicine is not only to increase the life span, but also to ensure that the additional years are efficient and full of joy. Antiaging is based on principles of weighed and responsible medical care that conform to approaches that have become firmly established in other spheres of preventive medicine. Antiaging is a clinical field with more than 30,000 practicing doctors working in 80 countries of the world (according to 2009 data). Antiaging medicine combines high biomedical technologies of diagnosis and treatment aimed at detection of diseases at the earliest stage and their most effective treatment. Computer tomography, ultrasound, and positron emission tomography; stem cells treatments; genetic engineering and nanotechnology are integral components of antiaging medicine. Antiaging medicine is the start of a new era in medicine that may lead to a real breakthrough in the provision of longevity, on a scale which dwarves all achievements in medical history.

Nowadays, the application of most "antiaging" measures is based on the simple but reasonable suggestion that all biological processes attending aging are harmful. The therapeutic consequence of this suggestion is the fact that correction of any biological aging-related changes will have advantageous results and provide macrobiosis [10].

Aging-related oxidizing stress and the application of antioxidants are typical examples of such an approach. As early as 1956, Denham Harman's free radicals theory of aging [21] suggested that aging is associated with the increase of free radicals, mainly in mitochondria, that becomes apparent in changes of such markers of oxidative damage as lipoperoxidation. In 1961, Harman [22] and other researchers [23, 24] tried to retard aging using antioxidants or manipulating the genes that control antioxidant protection level. The results were inconsistent, but recent meta-analysis of clinical antioxidant testing on people (although it should be specified that none of the examined antioxidants was specially meant for retardation of aging) has shown that antioxidants do not increase the life span and may even increase risk of untimely death [25, 26].

Hormones have been tested as geroprotectors for a long time, and many hormonal biologically active additives are widely used for retardation of aging [27]. It is known that the level of

growth hormone (GH) decreases with age, and some features of aging (in particular, sarcopenia) are similar to those observed in people with GH deficiency [28]. It has been shown that application of GH in elderly people has some positive effects but there has been no demonstrated influence on aging [29–31]. It is interesting to note that both mice and people with damaged GH receptors have longer-than-normal life spans [32]. This circumstance supposes that it is GH deficiency, not substitutive therapy GH that allows for achievement of macrobiosis.

Similarly, for men, activity of testosterone in blood serum decreases in old age and that is why substitutive therapy by testosterone is common [33]. But clinical research has not been able to present convincing evidence of a health gain or the retardation of aging when using testosterone [34] and one recent study has been discontinued because of a higher rate of cardiovascular disorders among elderly men who were taking testosterone [35]. Application of dehydroepiandrosterone has also not shown any advantages for health in old age, despite the fact there is an obvious age-related decrease of this hormone [36]. Although it is well known that the levels of concentration and activity of many hormones decrease with age, Olsen and Everitt's data should not be ignored as they proved long ago that hormonal exhaustion of rats by hypophysectomy, paradoxically increases life span and retards age-related changes [37].

According to D. LeCouteur et al. [38], the argument that every biologic change attending the aging process is surely harmful, can be questioned. Some age-related changes can, in fact, represent by themselves useful adaptive reactions and favor the prolongation of life. For example, GH exhaustion [39] and oxidizing stress [40], which develop with age, can potentially increase life span. This questionable concept was named "adaptive senectitude." The therapeutic consequences of this concept aggravate existing "age-related peculiarities" [41]. Besides GH exhaustion, this therapeutic approach has not been realized yet but has a right to existence.

Role of intestinal microflora in the aging processes. Aging itself has a relatively insignificant influence on the gastrointestinal tract, but due to a decrease in adaptive capabilities of the gastrointestinal tract, elderly people do not recover easily from disease. A decrease in adaptive capability also decreases tolerance to medication. A reduction in time for gastric evacuation results in a higher satiation and higher risk of an unbalanced diet in elderly people. There are many theories that microflora of the gastrointestinal tract actively participates in the processes of an organism's resistance to diseases, and the fact is that the balance of intestinal microflora is influenced by unfavorable environmental factors and stressful conditions, including psychological ones. In such conditions, probiotics have a positive influence. There are a lot of scientific publications on the clinical research of probiotic preparations and the results are varied and represent both objective and subjective information. Although, it should be noted that all studies have been conducted with limited number of experimental subjects. At the same time, results showing decrease of lactose intolerance and cure of diarrhea are well-grounded.

Failure of digestion as a result of inadequate activity of lactose in the human intestine affects about 70% of population in the world. Lactose is recycled more effectively in yogurt than in milk which may be related to bacteria present in yogurt which, in thin intestine, produce required enzymes. In particular, it has been proven that consumption of products containing *L. acidophilus* promotes digestion of lactose, due to the production of ß-galactosidases. Lin and coauthors have shown the importance of selecting strains that are capable at relieving symptoms of lactose intolerance. Nevertheless, the number of studies demonstrating application of probiotics and synbiotics as additional therapy in elderly populations is limited.

Induced pluripotent stem cells and aging (IPSC). Recent achievements in the study of the human genome have created new foundations for development, which in the near future will become preventive and personalized. This will increase considerably the arsenal of options used for preservation of health and the struggle against diseases, and, consequently, will further improve prospects of life extension. The discovery of new unique properties of stem

cells gives practically unlimited opportunities for the restoration of organs and lost functions by substituting damaged or "worn" cells with new ones. This area of study is called regenerative medicine and its capabilities, in fact, are the key to immortality. In order to effectively use promising achievements of biomedicine and select the most promising areas of scientific research, it is important to understand what goes on during aging and its biological mechanisms. There are methods of using a combination of external (environmental) factors and internal (genetic) factors, which makes it possible to cause the phenotype of neurodegenerative diseases of cells—derivatives of IPSC. As degenerative diseases are often a principal part of aging, a legitimate question arises: can we model aging itself using IPSC? If yes, we will be able to study aging at levels that are not available for study right now because of the limitations of conducting research on humans, and the inadequacy of animal models. Taking into account the potential of IPSC cells, we will be able to study aging of an organism in dozens of various types of cells, by various physiological processes, and idenfiy the influence of various external factors. Studing the aging cells of the heart, brain, liver, and pancreas in culture would allow for the real-time study of cellular aging and mechanisms involved. But, as is known, the more complex a disease is, the harder it is to model. Aging as a multifactor process will present a certain complications for modelling. However, the unlimited amount of cell material which, in theory, IPSC technology presents, will allow the gathering of the required amount of material for research on different theories of aging and also their combinations. Development of tissue engineering will make it possible to study processes of the aging on tissues and, perhaps, entire organs.

Thus, despite the abovementioned limitations, the possibilities of IPSC in the modeling of aging processes, the study of factors which influence aging, molecular mechanisms of aging, markers of aging, and the theory of aging can be called promising.

Technology based on the effects of a calorie-restriction (CR) diet. It is known that reducing caloric intake by 20% to 50% increases the life span of many species, from yeasts to primates [42, 43]. Despite the presence of controversial results, nowadays CR therapy has been acknowledged as the main focus of experimental antiage therapy [44–48].

Due to complexity of keeping a CR diet in people, there have been numerous attempts to develop preparations modeling the CR-effect—calorie-restriction mimetics (CRM) [49]. For development of CR mimetics, usually four main targets are used: insulin-like growth factor-1 (IGF1), sirtuins, mTOR, and 5′ adenosine monophosphate–activated protein kinase [50–53].

It should be underlined that, so far CR and CRM approaches to life extension have not been discredited. This allows, in a certain way, a narrowing in the search for new potential geroprotectors and a new focus on the search for analogs of resveratrol, metformin, and rapamycin.

Despite the introduction of promising genetic technology, achievements in proteomics, and highly efficient screening, so far there has been no remarkable progress in the search for new biologically active medical substances, the creation of medical products, the principles of new drug development.

To a considerable degree, the biology of aging is an unmastered field vis-a-vis the application of search principles and the creation of new medical products with geroprotector potential. Any effective medical product impacting the aging process will undoubtedly become a "blockbuster" drug, because the target group of consumers potentially includes everyone. Besides, the profit which is now generated by antiaging supplements clearly demonstrates that people are ready and eager to pay for the retardation of aging. Thus, there are lots of strong reasons why biogerontology and the search for geroprotectors shall be the focus of researchers' attention.

The first step in developing medical products is the selection of a target or the identification of the purpose of the medical product [54]. Developing new medical targets on the basis of

aging biology is a first-priority task. As soon as the target has been chosen, the next step is to conduct screening. Existing libraries of chemical and natural biologically active compounds with activity regarding a certain target are used. There are many commercial libraries of this type, including those containing combined and natural products, with over 1 million various compounds. It is assumed that among them there may be at least 10,000 potential geroprotectors [55]. In addition to commercial libraries, academic collections are at our disposal—for example, a rather big bank of original domestic phytosubstances their semisynthetic derivatives and synthetic analogs including poliphenols, flavonoids, secsviterpens—which were created in the Republic of Kazakhstan at the international scientific holding "Phytochemistry".

Determining experimental models for the testing of geroprotector activity and estimating their effectiveness is the next global task when creating a "drug discovery technology" system. Absence of a generally acknowledged model for evaluation of geroprotectors requires planning a comprehensive testing program using models from subcellular structures (for example, "old mytochondria"), lower fungi—nematodes and fruit flies to mammals (rodents with accelerated aging).

With favorable screening and conduction of preclinical estimation of toxicology and pharmacology, candidate compounds with geroprotector potential shall appear. The next natural stage is the clinical research of geroprotectors. Estimating the influence of any biologically active substance on aging requires the presence of end points, which shall be life span and the appearance of aging-associated diseases, or so-called "surrogate endpoints," for example, biomarkers of aging.

Evaluation by end points is, of course, a problem because studying them requires conducting very long studies, especially for higher order animals or humans.

So far, the real solution is applying aging biomarkers [56]. However, there has been no accepted set of biological and clinical biomarkers of aging [57]. It is assumed that adequate biomarkers of aging shall meet these criteria:

- They shall predict rate of aging and be better at predicting life expectancy than chronological age on its own;
- Control and represent the main process underlying aging, not consequences of a disease;
- Be available for repeated verification and estimation with no harm to humans; biomarkers should work both for humans and laboratory animals.

Biomarkers such as the delay of age-related physiological changes and the appearance of associated diseases, and not aging itself, can be adopted as basic biomarkers [58]. But laboratory biomarkers, or rather their combination, shall be an obligatory addition.

Thus, possible sets of biomarkers can be envisioned as combination of basic indicators, characterizing physiology of humans or laboratory animals and representing the most obvious aspects of aging and additional laboratory indicators which can be considered as molecular markers.

Considering the significance of functional results—for example, acivities of daily living and independence in everyday life for elderly people—it is quite possible that when testing geroprotectors, a number of clinical markers will also include muscle and intellectual weakness, the need for external assistance, and degree of dependence.

6.4 MAIN DIRECTIONS OF THE MOST PROMISING RESEARCH

An analysis of numerous biological theories of aging indicates significant changes in researchers' views based on achievements of science and practical activities. Purely theoretical and hypothetical speculations move toward and evolve into scientifically proven conceptual approaches

in theories of aging. It is the scientifically proven approach that makes it possible for a lot of authors to suggest or assume specific measures for longevity improvement and prevention of aging-associated diseases. It should be particularly noted that the major achievement of the last decade lies in the conceptual understanding of aging not as a fatally programmed process of organism damage and senescence, but as a comprehensive and complex process of adaptation with complex regulatory changes. Controlling these regulatory mechanisms can prevent the development of pathology that aggravates aging, and improve the health of old people. If early theories of aging were based on reviews of organs or systemic disturbance then the subsequent theories have been based on cellular, molecular, and genetic mechanisms. The latter does not support the distinction between biological theories of aging, but rather it supports the complimentary approach to integrating the theories taking into account the current lack of integrated theory of reproductive development and aging, which could be most fully capture the variety of system processes that lead to different types of aging, as well as to substantiate possible integrated programs for improvement of quality longevity and anti-aging. Development of an integrated concept of reproductive development and aging, in our view, has great scientific and practical importance, particularly given the current achievements and new knowledge in genomics, proteomics, and regenerative medicine.

The integrated concept of reproductive development and aging, based on the characteristics of synergistic and antagonistic interaction of various internal and external processes, that have previously contributed genetically and phylogenetically to determined reproductive formation and development of human body, while counteracting the various effects of endogenous and exogenous character, presume redevelopment of evolutionally formed regulatory processes of reproduction towards specialization on the processes of adaptation of entire organism to internal and external impacts at the decline of reproductive function.

The loss of some functions can be offset by enhancing and reprogramming other functions and methods of signal transmission. Subsequently, natural failures during postreproductive ontogenesis can facilitate either enhancement or weakening of the processes, which then results in various types of aging. Analysis of the characteristics of imbalanced processes that lead to sustainable reproductibility and its gradual decrement answer a number of questions concerning human ontogenesis and biological aging evolution in particular.

At the present time there are three technologies which, in our opinion, are the most promising in the field of antiaging medicine and rejuvenation: usage of embryonic stem cells or pluripotent stem cells; usage of pharmacological substances (less promising); and usage of tissue substrate or hormones (even less promising) [59].

Presumably, research in the following directions will lead to significant advancement in the field of rejuvenation. Examples can be found in a number of research papers, some of which are listed here.

1. Reprogramming of adult differentiated cells and achieving a more pluripotent state by means of cell nucleus transfer, somatic cell nuclear transfer (SCNT), or expression of specific transcription factors, creating so-called induced pluripotent stem (iPS) cells [50]. It is certain that telomeres regenerate during nuclear reprogramming. It has been demonstrated that the structure of the telomere is dynamic and is controlled by epigenetic programs that can be changed by reprogramming [61]. It is believed that, after reprogramming, cell retrodifferentiation and rejuvenation can offer immense opportunities for tissue repair and cell rejuvenation; however, these measures may have risks of carcinogenic stimulation [62].
2. Application of pluripotent stem cells (PSCs) is the principal trend of cell therapy in the antiaging and rejuvenation field [63].
   a. Regenerating mitochondrion by means of induced pluripotent stem cell (IPSC) technology affords achievement of the "biological clock stoppage" effect [64]. Rejuvenation

of mitochondra, for example, by means of acetyl carnitine [65] can restore the antioxidant potential inherent in young cells.
   b. Usage of mesenchymal stem cells (MSCs) plays a significant role in the rejuvenation of tissue and restoration of their functions [66].
   c. Transplantation of autologous hematopoietic stem cells could possibly fully restore functionality of the peripheral nervous system in case of autoimmune damage [67].
3. Restoration or rejuvenation of the immune system by the resumption of DNA repair in T lymphocytes [68, 69], functional recovery of B lymphocytes, and B cell rejuvenation [70].
4. It is proven that the reactivation of telomerase in adult male mice not only stops aging, but actually provides a welcome proof of the principle that it is possible to rejuvenate the body tissue of mammals [71].
   a. Reactivation of telomerase as a means of treating diseases associated with a person's biological aging and as a method of rejuvenation [72].
   b. The search for telomerase activators is a highly promising research topic [73].
5. Hormonal drugs for antiaging and rejuvenation.

"Hope never springs eternal more, it seems, than when it comes to rejuvenation" [74]. The main debate concerning the use of drugs for rejuvenation revolves around testosterone, estrogen, growth hormone, and thyroid hormones.

In accordance with the provisions of The American Academy of Anti-Aging Medicine's Official Position Statement on The Truth About Human Aging Intervention [75]:

- Dignity is an actually detected outcome of rejuvenation.
- Disadvantages are many adverse drug reactions and side effects.
  a. The significance of somatotropic hormone is demonstrated in both aging laboratory animals [76] and clinical studies [77].
  b. The use of progesterone, estradiol, and testosterone is the subject of numerous clinical studies conducted under the auspices of the American Academy of Anti-Aging Medicine with mixed results and planned in accordance with The A4M Twelve-Point Actionable Healthcare Plan [78].

The use of different antioxidants in the experiments of mitochondrial oxidative stress has mixed results (e.g., α-lipoic acid, L-carnitine, and PMX-500F model of aging caused by D-galactose with no effect [79], polyphenol antioxidants, curcumene, and resveratrol with pronounced effect). Although one gets the impression that there is not enough clinical evidence to justify the use of drugs for rejuvenation in modern medical practice so far, research in gerontology suggests that an increase in longevity, and especially during healthy period of human life, is an absolute good for humankind. Previous approaches are inadequate for the maximum increase of human life and healthy longevity. New knowledge made it necessary to develop other gerontological approaches.

The approaches to gerontology studies, which were proposed by leading gerontologists at the summit of the U.S. National Institute on Aging (Sierra, 2009), are summarized into two kinds: priority and engineering approaches. Among these approaches, based on the actual situation at Nazarbayev University and the "Center for the Life Sciences," we chose to focus on the search for quality health markers and biomarkers of aging.

The set of biomarkers coincident with the testing objective to reveal the potential antiaging medicines and antiaging technologies by means of experimental models (cell cultures and laboratory animals) and humans (healthy volunteers and patients with "age-related diseases") is meant to be the result of the forthcoming research.

Among the features of the engineering approach, also designated by the National Institute on Aging, is the principle of screening the candidate pharmaceutical compounds and technologies

aimed at sirtuins, genes that regulate lifespan in lower organisms, and which are presumably important for human life expectancy. The main target is calorie restriction, since it is the only technology with the proven impact on life expectancy of many organisms, including mammals and humans [80–83]. Thus, identifying markers of aging, detecting hormesis markers, searching for mimetics of calorie restriction, and screening large numbers of synthetic and natural compounds for the ability to simulate calorie restriction presumably will be the content of upcoming studies.

The art of medicine exists in the synthesis of various observations that are often quite variable and poorly defined. Issues of identifying hormonal and metabolic factors, enzymatic and biochemical factors, as well as damage to the genome that plays an important role in gerontogenesis are very relevant. A well-known phenomenon discovered by Hayflik, the Hayflik limit, is the accepted model of in vitro cellular aging research. At the same time, the development of similar models for in vivo studies is of considerable practical interest. New scientific approaches are essential for the study of role of the gastrointestinal tract and intestine-endocrine system, while taking into account individual characteristics of the intestinal microflora [84–86].

For future clinical studies, as well as the development of effective innovation in GerontoteCHnology and geronto-engineering (genetic, cellular, and molecular regulatory intervention in target areas), it seems appropriate to conditionally separate the following age groups, depending on optimal health status: (a) compensated gerontogenesis, 65–75 years; (b) subcompensated gerontogenesis, 76–85 years; and (c) decompensated gerontogenesis, ≥ 86 years. Again, it should be emphasized that these age groups are conditional and some individuals may be subject to significant fluctuations. At the same time, the impacts of GerontoteCHnology and geronto-engineering will depend on the state of optimal health of elderly people. Under compensated gerontogenesis it is seen as appropriate and necessary to use various developments of geronto-engineering and GerontoteCHnology. Subcompensated gerontogenesis involves using substitutional developments of geronto-engineering and gerontotechnology, and in decompensated gerontogenesis it is mainly of a palliative nature.

In conclusion, it is necessary to highlight that at this stage the main strategy consists not only of understanding the biological nature of aging, but also in developing a methodology to affect and slow the processes of aging. The primary strategy of prolonging life can obviously consist of available antiaging methods, in the hope that future achievements of science and medicine will solve many problems more effectively and in a profound manner.

Based upon the analysis of available data and the results of studies in the field of antiaging and rejuvenation, and as stated in the preceding sections of the book, as well as upon prognostic conclusions of leading experts in the field of gerontology, we tried to present some concept of declaring a desire and ability to participate in the process of studying the proposed regulatory interventions in the process of human aging and antiaging and correction of the process.

The concept, in an ideal scenario, should be the basis for scientific research, with a goal to establish a scientific basis for providing quality longer life to the population of the Republic of Kazakhstan.

6.5 CONCEPT FOR THE DEVELOPMENT OF RESEARCH IN THE FIELD OF HEALTHY AGING AND ANTI-AGING UP TO THE YEAR 2020

6.5.1 Background

In recent years, many countries, including Kazakhstan, have been experiencing an increase in the proportion of elderly persons in their populations. According to the US Census Bureau, the number of Americans aged 85 or older has now reached 3.3 million and this population

is expected to reach 18.7 million by 2080. At the beginning of 2009, the number of retirees in Kazakhstan was 1.6 million, of whom 1.3 million were over 65 years of age. It is estimated that by 2030, 11% of men and 12% of women will be in this category in Kazakhstan. According to data from the Ministry of Health of the Republic of Kazakhstan, the life expectancy in 2009 in Kazakhstan was 68.6 years.

According to analyses by the UN, a state is considered to be an "older" population when the proportion of elderly persons exceeds 7%. Currently, in the Republic of Kazakhstan, the proportion of persons older than 65 years of age is 7.8%. Children under 15 years of age represented 24% and persons aged between 15 and 65 represented 68.2% of the total population.

The problem of population aging is a significant issue of concern amongst policy makers and health care leaders in Kazakhstan. The economic and social problems that an aging population brings are significant and therefore policies and research need to be directed towards solving and preventing these problems. President N. Nazarbayev has said that there should be efforts to develop programs in the field of anti-aging, as one means to face the challenge of the aging population in Kazakhstan. This Concept is an introduction to the vision and preliminary objectives of the Center for Life Sciences for its research and development strategy in the areas of healthy aging and anti-aging.

6.5.2 Vision & objectives

Vision

To establish the scientific basis for the study of healthy aging in Kazakhstan and develop a system for managing biomedical research in this field. This research will be used to assist the government in developing policies that improve the quality of life of the citizens of Kazakhstan.

Overall Goal

To develop capacity to pursue research in the field of healthy aging, according to international standards.

Objectives

- Establishment and development of the necessary infrastructure and human resources within the Center for Life Sciences;
- Train scientific researchers, professors of medical schools and public health practitioners in collaboration with foreign partners;
- Design research programs for the study of healthy aging and anti-aging according to international standards. Pursue the development of innovative geronto-technologies and geronto-engineering, in accordance with international standards;
- Develop new research methods for studying pathology of biological aging; develop predictive approaches to biological aging and determine the clinical effectiveness of interventions in these fields;
- Development of the process of education and research in the field of healthy aging and use this to develop an evidence-based medicine model of healthy aging and anti-aging;
- Integration of findings of research into clinical practice. Develop recommendations to inform policy the national level, in order to improve the quality of life of elderly people in Kazakhstan.

6.5.3 Mechanisms for Implementation of the Concept

The Concept will be implemented in the following manner:

- Improvement of legal and regulatory structures for the management and introduction of research findings on healthy aging and anti-aging;
- Development of research infrastructure and development of funding mechanisms for research in the Center for Life Sciences;
- Development of systems for training and assessment of researchers, professors of medical schools and health care practitioners, according to international standards;
- Develop systems for quality assurance of scientific research;
- Development of recommendations for effectively integrating the research results into clinical practice.

6.5.4 Phases of Implementation

At the outset, the following goals will be pursued (2011–2013)

- Perform analyses of the current legislation related to the management of research projects in the fields of healthy aging and anti-aging;
- Perform analysis of the medical and demographic characteristics of the elderly population in the Republic of Kazakhstan;
- Establishment of a database of long-lived persons of the Republic of Kazakhstan and develop a program for the comprehensive study of risk factors of pathological aging;
- Develop expertise related to biomarkers of aging, their relevance, their sensitivity and specificity;
- Develop and understanding of the influence of pharmacological compounds on biomarkers of aging.

Organizational Actions during the first phase

- Establishment of systems for quality control of research, based on international standards for laboratory, clinical and scientific research;
- Development and equipping of the Center for Life Sciences laboratory facilities;
- Development of an institutional financing scheme;
- Development of training standards for scientific staff, professors of medical schools and health care practitioners. Provide training to staff in leading educational institutions in the area of healthy aging and anti-aging.

Development of scientific and research programs on the following topics:

- Study of biomarkers of aging at the physiological, cellular, molecular and chromosomal level;
- Use of embryonic stem cells or pluripotent stem cells for anti-aging;
- Use of pharmacological substances for anti-aging and rejuvenation.

During the second phase of development (2014–2016) the following goals will be pursued

- Introduction of there search results into the training programs of medical school sat the graduate and post-graduate level;
- Development and implementation of preclinical studies and clinical trials of innovations in the area of geronto-technology and geronto-engineering;
- Engage with international experts to analyze impact of ongoing scientific research.

During the third phase (2017–2020), the following goals will be pursued

- Clinical studies carried out within the framework of research projects in accordance with good clinical practice;
- Introduction and implementation of the healthy aging and anti-aging research results into clinical practice;
- Development and commercialization of innovative technologies in the field of healthy aging and anti-aging;
- Transfer of biomedical technologies developed at national, regional and international levels;
- Active involvement in developing national policy in the field of gerontological research and anti-aging, with the purpose to improve the quality of life of elderly persons;
- Implementation of research programs and key performance indicators to measure the impact of research findings.

6.5.5 Expected Results

The following results are anticipated as a result of implementing this Concept

- The CLS will have established modern institutional infrastructure. The CLS will be staffed with trained researchers in theoretical and practical aspects of the science of healthy aging and anti-aging. These researchers will be able to conduct research and implement the results in accordance with international standards;
- The CLS will have developed an international network of leading research partners in the area of healthy aging and anti-aging;
- The CLS will have developed a database of elderly persons in Kazakhstan and created the necessary software for maintenance of this database;
- The CLS will have developed staff with an understanding of biomarkers of aging and who are able to apply this understanding in research and clinical practice;
- The CLS will be developing geroprotectors with a goal to use them to increase quality of life and promote healthy-aging;
- The CLS will be developing new models for biomedical methods of research used in the field of healthy aging;
- The CLS will be developing means to evaluate policy in the field of health aging at the national level;
- The CLS will be contributing to the improvement of the quality of life of the elderly population in Kazakhstan by dissemination of developed technologies throughout the country;
- The CLS will commercialize the results of research projects in the field of healthy aging and anti-aging.

Implementation of the present Concept will bring positive change to scientific research and to public policies in the field of healthy aging and anti-aging. The goal is to improve the quality of the medical research programs and projects to international standards. Evidence-based recommendations will be developed for the establishment and development of gerontological care services for the population of the Republic of Kazakhstan. This will have positive impact on quality of life, increased life expectancy and the ability of elderly people to make significant contributions to society.

6.5.6 Summary

Research priorities in the field of healthy-aging must be informed by an understanding of the demographic trends in Kazakhstan. Statistics indicate that the population of Kazakhstan is aging, using conventional demographic definitions. The CLS is pursuing research in the area

of healthy-aging with an appreciation that elderly people make a significant contribution to the development of Kazakhstan. The strategy of the CLS includes pursuing research that deepens our understanding of the mechanisms of the aging process and also contributing to the development of interventions that delay or halt aging processes. Foundations have been laid for international partnerships that have potential for significant contributions in the field of health aging and anti-aging. The CLS desires to be a partner and contributor in this field in the years to come.

This concept was developed in accordance with:

- Code of the Republic of Kazakhstan dated September 18, 2009 "The people's health and the health care system"
- Law of the Republic of Kazakhstan dated January 19, 2011 On the status of "Nazarbayev University" "Nazarbayev Smart Schools" and "Nazarbayev Fund";
- Law of the Republic of Kazakhstan dated February 18, 2011 "On science";
- The State program of forced industrial-innovative development for 2010–2014, approved by Decree of the President of the Republic of Kazakhstan dated March 19, 2010 No. 958;
- State Program for Health Development "Salamatty Kazakhstan" for 2011–2015, approved by Decree of the President of the Republic of Kazakhstan on November 29, 2010 No. 1113;
- National Programme for the Development of Science and Education for 2011–2015, approved by Decree of the President of the Republic of Kazakhstan dated December 7, 2010 No. 1118;
- Strategic Plan of the Ministry of Health of the Republic of Kazakhstan for 2010–2014, approved by the Government of the Republic of Kazakhstan on February 10, 2010 No. 81;
- Concept of reforming the medical science of the Republic of Kazakhstan for 2008–2012, approved by order of the Minister of Health of the Republic of Kazakhstan on February 19, 2008 No. 79;
- President's message to people of Kazakhstan on January 29, 2010 "New Decade – New Economic Growth – New opportunities of Kazakhstan";
- Strategy of JSC "New University of Astana" in 2010–2012, approved by the Board of Directors of the University on October 17, 2009

6.5.7 Indicators of Research Impact

1. **Infrastructure Development:** Capacity of the laboratory infrastructure of the Center for Life Sciences to contribute to domestic and international research projects.
2. **Training and Human Resources:** Number of researchers affiliated with the Center for Life Sciences who have received or who are receiving training (including graduate or post graduate studies) in target areas of research with international research partners.
3. **International Collaboration:** Creation of an International Expert Advisory Board. Number and duration of formal partnership with leading academic and research institutions.
4. **Knowledge Production:** Number of publications resulting from research in the Center for Life Sciences. Impact of those publications.
5. **Informing Policy and Practice:** The extent to which the work of the Center for Life Sciences has influenced government policy and clinical practice guidelines.
6. **Cost Savings and Cost Recovery:** Estimates of costs savings achieved through implementation of technologies developed through the Center for Life Sciences research programs. Number and nature of patents, spin-off licenses and companies from intellectual property generated from research of the Center for Life Sciences. Income from intellectual property commercialization.

CONCLUSION

It is quite difficult and often a thankless task to make predictions in the area of science of human aging and the related practical problem of extending human life. For example, in the 1960s, a large group of American scientists, working for RAND corporation, predicted a 50 year increase in life expectancy by 2020. Many members of that study had already abandoned their prediction. Nevertheless, research results established an actual scientific basis for the possibility of controlling the processes of aging and developing technologies to extend life.

This possibility is supported by three arguments: (1) nature itself created diversity among species' life expectancy from a few hours to hundreds of years; (2) within each species, including the human population, there are amazing examples of longevity (i.e., there is possibility of aging differently); and (3) experimental gerontology approaches that may increase the individual life span by 30%–50%. Modern achievements and possibilities of medical and biological research open new horizons of promising scientific direction. The first results of these studies allow us to speak about controlling the processes that lead either to a long life or pathological aging as well as about the technologies that form the basis for development of antiaging medicine and ensuring quality longevity. There is no doubt that new opportunities of gerontotechnology and geronto-engineering will improve practical effectiveness of research in longevity and quality of life. These studies made it necessary for analytical generalization of data, which are covered in the suggested book and present the initial stage for the further elaboration in the field of qualitative longevity and anti-aging

It should also be noted that the main strategy at this stage is not only to understand the nature of aging and to identify integral biomarkers, but also to develop a methodology to control the processes that lead to pathological aging and eventually slow them down or stop completely. It is obvious that the primary strategy for extending quality of life can consist of ongoing improvement and application of available antiaging methods, in the hope that the further development of medical science and practice will comprehensively solve many problems.

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CENTER FOR LIFE SCIENCES

The NU Center for Life Sciences (CLS) was established in December 2010 as a private entity owned by Nazarbayev University. The CLS strives to transform medicine and healthcare in Kazakhstan through innovative scientific research, rapid translation of breakthrough discoveries, educating future clinical and scientific leaders, advocating and practicing evidence based medicine, and pursuing research in personalized and predictive medicine. The underlying goal of all these activities is to improve health and quality of life.

MISSION

To develop fundamental science and discover new knowledge about the nature and behavior of living organisms, as well as application of that knowledge to improve quality of life and longevity, reducing the burden of disease in humans.

VISION

To improve the quality of life, health and longevity through the practical implementation of modern advances in biomedical science to clinical practice, as well as the establishment of sustainable scientific and legal structures for a competitive biomedical industry, with a subsequent contribution to the diversification of the Kazakhstan economy

MAIN AREAS OF RESEARCH

- Genomics and Personalized Medicine;
- Regenerative Medicine, Bioengineering & Artificial Organs;
- Tissue Bioengineering, Transplantation Medicine and Innovative Cell Technology;
- Translational Medicine and Healthy Aging;
- Global Health.