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Melatonin, Aging, and Age-Related Diseases

Perspectives for Prevention, Intervention, and Therapy

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The high incidence of age-related diseases in the increasing population of elderly people has stimulated interest in the search for protective agents that have the capability of preventing premature aging and delaying the onset of degenerative disorders. To preserve health in old age becomes a primary goal for biomedicine, because the increasing longevity in our societies is associated with a rise in morbidity. The difficulties in finding new approaches and safe strategies for prevention, intervention, and treatment are related to the lack of theoretical background as well as to insufficient models to test the efficacy of anti-aging agents. Melatonin is a prime candidate for slowing the aging process and targeting its underlying pathology. Melatonin has profound gerontoprotective and antioxidant activities. Because enhanced oxidative stress plays a crucial role in the aging process and chronic diseases associated with senescence, the adminstration of a potent amphiphilic antioxidant agent with high bioavailability such as melatonin may become a promising, safe, and effective intervention strategy to slow aging and the initiation and progression of age-related disorders. Investigations on melatonin and its anti-aging activity may be of great benefit in increasing life quality of the elderly.

Key Words: Adaptogen; aging; antioxidant; life span; melatonin; neuroprotection.

Introduction

During the past decade numerous reports, sometimes contradictory, have appeared concerning the role of melatonin in aging, and some publications have demonstrated very potent gerontoprotective actions by the indoleamine, most of them related to the prevention of age-associated oxidative stress and damage *(1,2)*. Melatonin has a broad spectrum of physiological effects *(3)*, including, but not limited to chronobiological, immunomodulatory, neuroendocrine, and antioxidant activities, which could all contribute to the observed antiaging potency of this natural agent.

Melatonin and its metabolites act as catalytic antioxidants to safeguard mitochondrial electron transfer reactions and thereby increase energy metabolism efficacy, as proposed more than a decade ago *(4)*. Recently, the effects of the indoleamine on mitochondrial oxygen and energy metabolism have been in the focus of work in the field of experimental gerontology.

Aging is a multicausal process leading to a decline of maintenance mechanisms and an exponential accumulation of molecular damage resulting in degeneration and dysfunction at the cellular and organismal level. Old organisms generally exhibit a dramatically reduced adaptive potential and they are unable to cope with a variety of stressors and adverse stimuli *(5)*. Senescence refers to those postmaturational changes that underlie the increasing vulnerabilities to challenges, thereby decreasing the ability to survive.

With advanced age and the onset and progression of age-related diseases, the predominantly nocturnal synthesis of melatonin decreases significantly in various species, including humans *(6)*. The rate of aging and the time of onset of age-related disorders can be retarded by melatonin supplementation or treatments that preserve the high amplitude of the endogenous rhythm of melatonin formation *(5)*. The amplitude of the diurnal rhythm of melatonin in the elderly is reduced and in some neurodegenerative diseases such as Alzheimer's disease almost abolished *(7)*. The disturbed circadian melatonin rhythm may have profound effects on the health and well-being of the elderly.

Many important physiological processes such as the sleep–wake cycle, the core body temperature, performance, alertness, and secretion of many hormones, to name only a few, exhibit a profound circadian rhythmicity in healthy young organisms. Such rhythms may play an important role in maintaining health and well-being. Advanced age is characterized by a deterioration of the overt circadian patterns of these rhythms with reduced amplitudes, timing problems, disorganization of temportal order, and loss of entrainment stability and responsiveness to Zeitgebers *(3)*. Several findings suggest that with advanced age and, even more so, with different age-related diseases there is a general desynchro-

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nization of overt rhythms based on dysfunction and loss of control by the suprachiasmatic nucleus *(8)*. Melatonin acts as a Zeitgeber *(9)*. The indoleamine may therefore exert beneficial effects in terms of aging because of its effects on the circadian timing system.

It has been suggested that a periodic administration of melatonin may readjust and reset the phase, thereby synchronizing internal rhythms and increasing their amplitude to normal in old organisms *(9)*. Although many theories have been proposed relating the pineal gland and melatonin to aging and its pathology, the decline in circulating melatonin over the life span, which is associated with a general decline of many circadian rhythms with advanced age, is a good indicator that even physiological concentrations of melatonin may play an important role in this process.

Although recommodations for melatonin supplementation in the elderly should be considered carefully as a strategy for prevention and protection in the increasing population of elderly people, there is an urgent need for extensive studies evaluating the efficacy and safety of the long-term adminstration of this endogenous indoleamine and the development of guidelines for the use of this natural adaptogen and antioxidant compound to improve the quality of life in advanced age.

As already mentioned, the aging process is undoubtedly complex and multifactorial. Melatonin and its kynuramine metabolites have multiple effects that may be beneficial for an aging organism such as the antiamyloidogenic, antiapoptotic, antiexcitatory, antiinflammatory, antioxidant, chronobiological, immunomodulatory, and neuroendocrine activities as well as the potent adaptogenic, sleep-promoting, and stress-relieving properties *(1,2,5,10)*. Performance and stress resistance can be enhanced by long term administration of this natural sleep promoting agent *(11)*.

All of these gerontoprotective effects may act in concert and can not only exert a significant increase in well-being, but also contribute to a reduced incidence and severity of some age-related disorders affecting the elderly *(1,2)*. Recent animal studies on melatonin, as mitochondrial medicine clearly indicates, show that melatonin at fairly low concentrations when given chronically can even act as a rejuvenating agent reversing and normalizing the disturbing effects of aging on the oxygen and energy metabolism in senescence-accelerated mice *(12)*. In this review, the broad evidence for melatonin as a potent preventive and protective agent will be presented in a concise manner and the future potential and use of this indoleamine, its kynuramine metabolites, and similar derivatives with important implications relevant to many disorders associated with the aging process are discussed.

Melatonin as an Adaptogen and Antioxidant

The pineal is a primary source of circulating melatonin *(13)*. Pinealectomized rats seem to have a reduced life span associated with a significant increase in oxidative stress and damage *(14,15)*. The administration of the indoleamine can extend the life span of mice, rats, fruit flies, and many other organisms, reduce age-related pathology and biomarkers of the aging process, as well as increase health and performance and induce a more youthful appearance of the treated animals *(16)*. Many studies have also demonstrated the inhibition of tumor growth by melatonin *(17)*. The discovery of potent immunomodulatory and antioxidant effects of melatonin has greatly stimulated the interest in these observations.

Several studies in the field of experimental gerontology have provided some understanding of the evolution of aging and developmental processes as well as the important impact of endogenous and exogenous adaptogenic agents, which allow the organism to anticipate and counteract environmental factors *(5)*. Early in evolution, molecules emerged to protect cells against dangerous challenges and stressors allowing for adaptation *(5)*. The selection pressure in favor of the evolution of endogenous mechanisms that increase the ability to survive even in adverse conditions has generated numerous different molecules that act as chemoprotective agents *(5)*. It has been proposed that indolic compounds, and in particular, melatonin, are involved in the regulation of electron transfer *(5)* and act as potent metabolism modifiers *(5)*. These powerful mediators of feedback between the external environment and internal milieu of living organisms may ensure that cells can cope with a variety of stressors and adverse stimuli.

However, contrary to the previously mentioned observations suggesting that melatonin is a potent agent in slowing the aging process and its detrimental effects on health and performance of organisms, several other studies at the same time have failed to demonstrate an effect of melatonin on life span or age-related pathology. Moreover, recently, some studies have even reported an enhanced tumor incidence despite a life span extension following chronic melatonin treatment in certain mice strains *(16)*.

The authors of the original publications have cautioned that the apparent enhanced cancer rate in the melatonin-treated animals is very likely a laboratory artifact owing to two factors: the enhanced longevity and the greater body weight of the experimental group, both positively correlated with cancer initiation in the specific strains used *(18)*. Nevertheless, these isolated observations have been taken out of context and misinterpreted as evidence that melatonin might be a potentially very dangerous substance. Based solely on these data, it was even suggested that long-term treatment with this gerontoprotector might be associated with an enhanced risk to develop cancer *(16)*. This claim is not totally unfounded, nor unexpected, because all such redox-active agents including the antioxidant vitamins C and E may principally increase cancer incidence by exerting detrimental prooxidant effects *(16)*. It is rather unlikely that melatonin promotes cancer initiation and progression because the great majority of animal and clinical studies

have clearly and unequivocally shown potent oncostatic effects of the indoleamine indicating a beneficial role of melatonin in combating cancer *(17)*. Moreover, all clinical studies to date find that melatonin slows the advancement of malignant diseases and epidemiological data suggest that lower melatonin levels are associated with a greater cancer risk *(17)*. This is certainly true for the melatonin deficiency due to senescence, because onset and prevalence of nearly all neoplastic diseases are positively correlated with aging *(17)*. In fact, numerous studies summarized in detail recently *(17)* have consistently demonstrated that melatonin treatment delays the onset and occurrence of a wide range of age-dependent diseases and disabilities, including cancer, reducing both tumor initiation and progression.

Antiaging medicine is any intervention that delays the development of age-related pathology and other adverse changes in old age, which lead to enhanced morbidity and mortality. The findings that melatonin can reduce the exponential increase in oxidative stress and damage associated with senescence are very robust and have been confirmed in many studies on laboratory animals. Extensive independent research by many different laboratories as reviewed previously *(1,2)* has strongly suggested that it is possible to retard the rate of aging as well as cancer initiation and progression by melatonin adminstration.

A controversy about melatonin as a protective agent as initiated a decade ago denouncing the claim about melatonin as medicine as hype *(19)* has now come to rest with the increasing flood of publications confirming the potent antioxidant effects of the indoleamine in increasing survival and health in a wide range of organisms *(1,2)*. Because age- and disease-related oxidative stress is frequently associated with mitochondrial dysfunction, only amphiphilic antioxidant agents of high stability, selectivity, and bioavailability that traget these organelles such as melatonin can provide the necessary on-site protection.

Recent findings indicate that such advanced mitochondrial antioxidants catalyzing one-electron-transfer reactions *(20)* have great potential as preventive and protective agents in supporting and preserving mitochondrial function by increasing oxygen and energy metabolism efficacy.

Giving the convincing evidence that melatonin can promote health, performance, and well-being in old age, the present state of knowledge based on solid experimental work in animals and humans is encouraging and invites future studies that explore melatonin supplementation as a safe and effective intervention strategy to slow, stop, or reverse aging and its pathology. The only open question that remains to date is: Do all the exciting observations and the firm evidence for prevention and protection from animal studies extend fully to the human use?

After a careful evaluation of the safety of long-term melatonin treatment with an intensive search for possible side effects, this endogenous antioxidant and adaptogen may slow, stop, and even reverse many of the devastating age-related diseases and their pathology that are based on an enhanced radical formation, oxidative stress, and mitochondrial dysfunction. In this regard, we have now firm evidence that melatonin adminstration can restore the age-dependent deterioration of mitochondrial status to a youthful level by reducing electron leakage as measured by nitroblue tetrazolium reduction, oxidant formation, and proton dissipation in animals as different as rotifers and rodents *(20)*.

Interestingly, similar findings in relation to oxidative energy metabolism in mitochondria with respect to superoxide anion radical formation have also recently been demonstrated in old ring doves, where melatonin also prevents and suppresses enhanced mitochondrial oxidant formation *(21)*. If the indoleamine is effective as a mitochondrial metabolism modifier even in birds, which are comparatively long-lived organisms, then this may indicate that the antioxidant should also work in humans to improve and support mitochondrial integrity and function. Moreover, recently, a first report of excellent quality has demonstrated that melatonin administration reversed age-dependent changes of gene expression in mice *(22)* and restored them to normal levels seen in healthy young animals.

Remarkably, melatonin's most pronouced genomic effects in mouse brain were seen on stress-related and proinflammatory gene expression with a complete restoration of the basal youthful profile *(22)*. Thus, dietary melatonin or its neuronal metabolites completely reversed age-related changes in gene expression *(22)*, and it is important to note that not only downregulation or decline of gene expresssion is affected by the indoleamine, but also the age-specific upregulation and senescence-associated enhanced gene expression. This is more important, because similar changes are seen in many degenerative diseases related to senescence, indicating that melatonin reduces the age-dependent increase in mortality mainly by suppressing the age-associated enhanced morbidity.

The antiinflammatory effects of indoleamines and kynuramines are a very imporant and rapidly expanding field of experimental work. Further areas of intense future research should include detailed studies of the effects of melatonin, its metabolites, and related molecules on mitochondrial oxygen and energy metabolism; determinations of the turnover of this indoleamine and measurements of kynuramines generated therefrom, in tissues and intracellular compartments, with an emphasis on the aspect that melatonin and metabolites act as catalytic antioxidants and possibly also as prodrugs; also a detailed exploration of the clinical pharmacology and toxicology of these endogenous biogenic amines should be carried out.

Theories Linking Melatonin with the Aging Process

In mammals, the biogenic amine melatonin is synthesized from the aromatic amino acid L-tryptophan in many tissues, but the circulating levels with the prominent nocturnal peak

are mainly of pineal origin *(6,13)*. Reduced melatonin concentrations with advanced age and even more so in age-related diseases such as Alzheimer's disease have been reported, demonstrating a high correlation to their progression and genotype, and, recently, these dramatic changes have been reviewed in detail *(6,7)*.

Extensive studies of many research groups have shown a reduction of melatonin formation and secretion in old animals and humans as well as a pronounced progressive deterioration of the diurnal rhythm in circulating melatonin leading to an almost complete absence of any detectable nocturnal peak as seen in many devastating degenerative diseases associated with senescence. The aging pineal gland and its reduced capacity to secrete melatonin in advanced age may have physiological consequences resulting in a melatonin deficiency status of senescent animals with highly detrimental effects on the old organism.

The decline in melatonin concentrations during physiological and pathophysiological aging have been studied extensively in the pineal gland, brain, and many other extrapineal tissues as well as in body fluids including, but not limited to, the cerebrospinal fluid, plasma, saliva, and urine *(6,7)*. Most studies have reported a highly significant decline in melatonin levels and the output of its main liver metabolite 6-hydroxymelatonin, with a few exceptions not showing a statistically significant difference being largely due to methodological reasons based on the large variability of nocturnal melatonin production and 6-hydroxymelatonin excretion and the timing of their age-related decline *(7)*.

Of great interest and importance in the context of this review are those recent publications which indicate that the maintenance of the circadian organization of melatonin formation may constitute an important marker for biological age and is of great potential use to probe the health status in humans *(23)*. Recently, these impressive findings associating melatonin with the aging process have been reviewed in great detail, covering all aspects of the topic including the few still controversial ones *(7)*. That not all questions concerning the age-related decline in melatonin have been successfully addressed and resolved becomes obvious when looking at a recent study reporting no dramatic changes in mice tissue melatonin and 6-hydroxymelatonin *(24)*. It is of utmost importance to investigate the turnover of the indoleamine and not to look only at the levels of melatonin, but also on those of the metabolites.

Collectively, the data are convincing that with advancing age less melatonin is produced and secreted thereby severely limiting the availability of this important health-promoting agent in animals and humans. It is thus not surprising that these observations prompted many theories linking melatonin to aging. Here we have to caution that coincidence must not be confused with causality, an often neglected problem that has been troubling experimental gerontology since its conception.

Early reports based on observations that melatonin administered in the drinking water to mice extended their life span and maintained the organism in a more youthful state were initiated because of the potent immunomodulatory effects of the indoleamine *(25,26)*. Recently, the researchers who published these findings in the late 1980s and early 1990s have modified and extended their hypothesis to suggest that the pineal gland and its secretory products (which may not be limited to melatonin) constitute an internal monitor and regulator of "self-control." According to their theory, aging is a consequence of a loss of "self-control" resulting in the breakdown of the immune system. They see a central role for melatonin and peptides released from the pineal in orchestrating the immune system.

Immunosenescence is often associated with autoimmune diseases and inflammatory processes. The latter can greatly accelerate the aging process. There are solid experimental data demonstrating an important immunoregulatory role for melatonin and other pineal potential secretory products in rodents *(25)*. The reduction in circulating melatonin levels may severely compromise this role and put the organism at risk of developing an autoimmune or inflammatory disorder. Melatonin and kynuric metabolites of the indole may be potent antiinflammatory agents as outlined below. Such effects may greatly contribute to the antiaging effects exerted by melatonin and its oxidative metabolites in counteracting the inflammatory pathology present in most elderly individuals.

Others view aging as a syndrome of a relative melatonin deficiency and a diminshed serotonin:melatonin ratio *(27)*. These researchers have presented some evidence that aging may be secondary to pineal failure in metabolizing serotonin to melatonin *(27)*. They suggest that serotonin is a proaging substance of great toxicity and have even called this melatonin precursor a "death factor." At first glance, this theory appears somewhat unusual. Nevertheless, some of their observations cannot be dismissed easily, such as the possible prooxidative actions of serotonin as well as some putative detrimental neuroendocrine actions of this important endogenous neuromodulator *(27)*. In this context, it is of interest to note that much speculation on the presence of a death trigger or an aging program have been put forward, which, contrary to the serotonin hypothesis, are immediately dismissed by the scientific community.

The existence of a central aging clock executing an internal developmental program that results in the elimination of the organism due to its death is another speculation that has survived for quite some time in the scientific community. The pineal gland has been proposed to be such an internal clock determining life span in coordinating a developmental program that culminates in the death of the organism *(28)*. The circadian melatonin signal would be the hands of this clock to inform all cells in the organism about the passage of time. Most researchers strongly oppose such views;

however, at present, we cannot completely rule out that such a biological program of cell or organismal death exists and we therefore have to consider the evidence without premature rejection.

Advanced age is characterized by a deterioration of circadian organization *(9)*. Chronobiologists have explored the important functions of circadian rhythms as well as their relevance to physiology and pathophysiology. Melatonin can act as an endogenous synchronizer and certainly can play an important role orchestrating circadian clocks. Initial reports have focused on melatonin as a chronobiotic agent with antiaging or life-extension properties *(9)*. Although some conclusions of the theory based on melatonin as an input to the master clock in mammals, the suprachiasmatic nucleus, are far fetched, many of the observations presented by these scientists to defend their theory are of great relevance and importance not only in chronobiology, but also in gerontology.

There is good reason to suggest that diminished melatonin secretion in old age may somehow be related to deterioration of many circadian rhythms and a dysfunction of the master clock in the suprachiasmatic nucleus. Furthermore, melatonin administration seems to improve sleep and is also capable of adjusting other important circadian rhythms in the healthy elderly and in patients afflicted with Alzheimer's disease *(11,29)*. Melatonin exhibits potent sleep-promoting effects by mainly reducing sleep latency *(11,29)*. Sleep disturbances are a common complaint of the elderly, and melatonin treatment has been shown to improve sleep efficacy *(11,29)*. The indoleamine has potent effects on other circadian rhythms as well, including the regulation of core body temperature, performance, alertness, and secretion of a variety of hormones *(3)*. Melatonin can induce hypothermia and, more importantly, may also reverse the age-dependent loss of the nocturnal drop in core body temperature experienced by many elderly, who are troubled by sleep problems. This could be of great importance in mediating at least some of the antiaging effects of the indoleamine. If melatonin acts as an internal Zeitgeber as proposed *(9)*, then a diminished signal would have profound detrimental effects on the organism.

The most recent theories on melatonin and aging are centered around the well-established antioxidant activity of the indoleamine, claiming that the melatonin deficiency with advanced age deprives the organism of an essential antioxidant to prevent and protect against oxidative stress and damage associated with mitochondrial dysfunction *(30)*. It is well known that the increased formation of highly reactive and toxic free radicals with age leads to an exponential increase in oxidative stress and damage, which may finally be lethal *(1,2,5)*. Although this view is strongly supported by much evidence, the conclusion that oxidative stress and damage causes aging may be premature. Here, we may have another good example for the confusion of coincidence with causality, because oxidative stress and damage could simply reflect a cofactor or even an epiphenomenon, not causally associated with aging.

Most of the current theories cannot sufficiently explain the role of melatonin in aging. Nevertheless, the most recent ideas may be promising and some interesting hypotheses have been put forward, which may guide us in exploring the link between melatonin and the aging process. It is very important that the theoretical background is sound and concepts are built on conclusive studies investigating the effects of melatonin on aging and vice versa. Although the interest is great in the potential causal association between the drop in melatonin in the elderly and the degenerative signs of aging, the research findings to date are highly suggestive but incomplete. Any claims of a causal relationship or even a proof of concept as often proposed in the non-scientific literature are premature. Although the age-associated loss of melatonin has now been known for decades and definite proof for the potent protective effects of the antioxidant is now available, the antiaging effects are not entirely explained and multiple mechanisms may be at work, some of them not yet well understood.

Evidence for Life Extension by Melatonin

The emphasis of the present review relates to the potential association between the reduction in melatonin and the consequential loss of its antioxidant and adaptogenic effects with age. Enhanced survival after melatonin treatment has even been observed in unicellular organisms and plant tissues such as leaves, fruits, and germ cells *(31)*. Apart from counteracting oxidants and other stressors, melatonin delayed senescence in monocots and dicots *(32)*. Additionally, the first evidence is available for a role of melatonin in regulating developmental processes such as dormancy and fruit ripening as well as germination *(31)*. In the unicellular protozoan, *Parameciun tetraaurelia*, the addtion of 43 μ*M* melatonin to the medium increased mean and maximum clonal life span by up to 25% *(33)*. Although other antioxidants such as vitamin C and E exerted similar effects, melatonin was by far the most potent substance ever tested in this simple model system somehow emulating the aging process observed in multicellular organisms.

The antiaging potential of melatonin has also been investigated in other invertebrates that serve as well established animal models. In aquatic rotifers, melatonin substantially increases mean and maximum life span, in both short- and long-lived species *(20,31,34)*. Very reproducible results on longevity have been recorded in many independent experiments using the bdelloid rotifer of the genus *Philodina*, as also described in greater detail in the section on age-associated oxidative stress. Owing to its potent antioxidant effects, the indoleamine is highly efficient in extending life span in this aging animal model, superior to all conventinonal anti-

oxidant agents *(20)*. To the best of our knowledge, the 50–100% increase in mean and maximum life span of this rotifer species is the most pronounced antiaging effect ever exerted by melatonin and was so far exceeded only by a single gerontoprotector, a recently developed amphiphilic nitrone *(20)*.

The advantages of the invertebrate animal model using rotifers as individually housed organisms of defined and uniform age are high throughput with extreme efficacy and low cost as well as time- and labor-saving protocols. In contrast to aging protocols using other organisms, the aquatic culture systems ensure that the agent applied is not lost and is fully absorbed by the treated animals giving highly reproducible and robust results not troubled by experimental artifacts. We have now firm and consistent evidence that melatonin and similar amphiphilic advanced antioxidants exert impressive antiaging effects and have remarkable gerontoprotective activities at low micromolar concentrations after chronic administration not matched by compounds lacking the superior bioavailability of these agents *(20)*. Because of the extensive nature of the studies involving many other laboratories, we have not yet been able to give a full report on these conclusive findings. The unique efficacy of amphiphilic antioxidants and their extreme high potency in enhancing rotifer survival, size, and fecundity indicate that mitochondrial dysfunction and oxidative stress are major determinants of longevity.

We have also seen consistent and promising results in extending mean and maximum life span by up to 100% in melatonin-treated water fleas. Life span extension by treatment with low micromolar concentrations of the indoleamine has been observed in the crustacean species *Daphnia magna* and *D. pulex*. In fruit flies, *Drosophila melanogaster*, we have observed significant increases in mean and maximum life span by 10–20% with chronic melatonin administration when fed at relatively high concentrations of 0.5–1.0 m*M* *(34)*. Higher concentrations are toxic, possibly by interfering with serotonin and *N*-acetylserotonin and the pteridine metabolism as well as sclerotization in these organisms. Because of the prelimary nature of the experiments on fruit flies and methodological problems with the exact application of the indoleamine, the findings have yet to be published.

A complete report on melatonin and aging in short- and long-lived populations of flies has demonstrated a high efficiency in life extension in short-lived animals, but revealed no effects or even a slight reduction in long-lived animals of this species *(35)*. Nevertheless, one recent full paper of excellent quality demonstrates highly significant effects in extending life span in *D. melanogaster* by chronic melatonin adminstration fully confirming and largely extending our earlier preliminary observations *(36)*. This work also demonstrates the antioxidant activity of melatonin by the impressive improvement in paraquat resistance of the flies pretreated with the indole *(36)*.

In contrast to the life-prolonging effects of melatonin in flies, water fleas, and rotifers, melatonin acutely shortened life span of *C. elegans (37)*. This effect has been reproduced in our laboratory and is due to the large amounts of melatonin and metabolites (50–100 μ*M*) taken up daily from consumed *Escherichia coli*, which serves as the food of this organism. If additional external melatonin is provided, more melatonin is converted by *E. coli* to 5-methoxytryptamine and 5-methoxyindoleacetic acid, the latter substance being a potent prooxidant toxin, which clearly shortens life span. Similar reductions in life span after adminstration of indoleacetic acid, but not indolepropionic acid and derivatives thereof, have also been seen in other organisms including rotifers, water fleas, and fruit flies. Interestingly, because all indoles, including melatonin as well as the indolepropionic and indoleacetic acids, act as growth factors, not only in plants, but also in the above-mentioned invertebrates, and can strongly increase cGMP levels, even in mammalian cell lines, the pro- or antioxidant effects seem to predominantly determine the effects of these compounds on survival and life span.

In mammals, the effect of melatonin on life span has been examined only in few studies with conflicting results. According to Pierpaoli, Regelson, and co-workers *(25,26)*, nightly melatonin supplementation in the drinking water at a low pharmacological dose of 1–2 mg/kg/d given to mice prolonged their life and preserved their youthful state. Impressive was not so much the rather small effects on life span, but the more youthful and healthy appearance of the melatonin treated mice *(25)*. The same group has done a number of independent experiments on different mice strains such as the BALB/C, C57BL/6, and New Zealand black demonstrating the gerontoprotective effects of nocturnal melatonin adminstration *(25)*. Chronic treatment with the indoleamine was started after the animals had reached at least middle age, indicating that melatonin may be given at an advanced age with success in slowing the aging process and its pathology *(25)*. Much less noted are the experiments in which melatonin, instead of enhancing longevity, reduced life span and increased cancer incidence, as most pronounced and significant in C3H/He femal mice *(25)*. A metaanalysis of the accessible data of this research group revealed that overall melatonin administration had no significant effect on life span of mice. This surprising result stands in sharp contrast to the many beneficial effects the authors observed after melatonin treatment.

In NZB/W female mice, Lenz and colleagues *(38)* also found that melatonin administered sc at a dose of approx 2–3.5 mg/kg at either 08:00–10:00 h in the morning or 17:00–19:00 h in the evening prolonged the life span of the treated animals. Melatonin protected against age-related damage in tissues including the kidneys and delayed the onset of diseases such as proteinuria *(38)*. Similar increases in survival were seen in male BALB/c mice after slightly higher concentrations of melatonin *(39)*. Interestingly, melatonin was most

effective after daytime administration, whereas Pierpaoli, Regelson, and co-workers *(25,26)* report otherwise, that only nocturnal administration was beneficial. Moreover, long-term admininstration of a pharmacological dose of melatonin, at approx 75 mg/kg with the diet orally, had no effect on life span as demonstrated in a large population of male C57BL/6 mice *(40)*. Interestingly, melatonin treatment was followed by either no or even a slight, but statistically non-significant, increase in tumor incidence in these studies on different cohorts of animals *(40)*.

To make the picture even more complex, in a large study by Oxenkrug et al. *(41)* on the putative antioxidant and anti-aging effects of *N*-acetylserotonin and melatonin in male and female C3H mice at a dose of approx 2.5 mg/kg/d given during the night with the drinking water, both indole antioxidants prolonged the life span of male animals by about 20%, but did not affect the life span of female mice. Both indole compounds increased and restored antioxidant capacity in old animals and dramatically reduced oxdidative stress and damage markers. Animals treated with *N*-acetylserotonin and melatonin exhibited luxuriant fur coats and appeared to be in much better health than the control mice.

Recently, melatonin was shown to increase both life span and tumor incidence in femal CBA mice *(18)*, when given with the drinking water during the night at a dose of approx 1–2 mg/kg/d chronically. Melatonin decreased body temperature due to a reduced metabolism and also increased body weight, possibly by a more efficient use of calories *(18)*. The authors also confirmed the highly potent antioxidant effects of melatonin in reducing many markers of oxidative stress and damage as measured in these old mice *(18)*. It is worth noting that melatonin administration at 0.1–0.2 mg/kg to female Swiss–derived SHR mice given with their drinking water during the night reduced body weight and spontaneous tumor incidence *(42,43)*. No effects were seen with higher concentrations of the indole at 1–2 mg/kg on tumor incidence or body weight *(42,43)*. Melatonin treatment significantly reduced tumor incidence and other age-related disorders in female HER-2/neu transgenic mice, but, in spite of its beneficial effects, the indoleamine slightly shortened rather than enhanced longevity in this mouse strain.

In the only study using rats as the laboratory rodent of choice and experimental animal model, Oaknin-Bendahan and co-workers *(44)* found that melatonin given with drinking water at night at a dose of 0.2–0.4 mg/kg/d to male CD rats resulted in a dramatic increase in 50% and 90% survival rate of the animals. These findings strongly suggest that melatonin may be beneficial and also highly effective in reducing age-dependent mortality *(44)*. Interestingly, a melatonin receptor antagonist of indole structure, administered at one-tenth of the dose of melatonin, had similar beneficial effects, and even augmented, when given together with melatonin, the already impressive antiaging effects exerted by melatonin treatment alone *(44)*. These observations indicate that membrane receptors do not mediate the gerontoprotective actions of melatonin in rats *(44)*.

Collectively, many reviewers of the current research have concluded that the findings linking supplemental melatonin to increased survival are not compelling for several reasons: (a) Many of the early observations and data are contradictory regarding the effects of melatonin on life span; sometimes even the same research group has published opposite results with melatonin increasing and decreasing life span. (b) The number of animals that have been used is small, the husbandry conditions were less than ideal, or the studies did not follow guidelines for long-term testing or principles of gerontological experimentation. (c) Until now, the total number of studies yielding conclusive results is limited. Unfortunately, even experiments of high quality have resulted in sometimes apparently disparate findings.

The data available to date are suggestive of an association between melatonin and longevity. More thorough investigations, however, are urgently needed to prove or disprove this relationship. To date it is not possible to resolve many of the still open questions addressed in this review. Nevertheless, this review will hopefully correct many popular misconceptions regarding melatonin and its effects on the aging process. Others issues will persist until new firm and consistent evidence by future solid studies and experimental work can be provided to give definite answers. The author strongly encourages an integrative approach, which is not solely focused on life span, but also includes other parameters reflecting the aging process. More enthusiasm for experimental gerontology will not be generated by repeating the mistake of doing only simple and flawed longevity studies involving large cohorts of animals suffering from chronic degenerative diseases. What is required are more innovative concepts and new animal models that provide faster and more reliable answers to the pressing questions of aging research.

In conclusion, it is necessary to stress that a critical review of the available data on the effects of life span by melatonin in rodents are derived from experiments that are fraught with many problems, pitfalls, and artifacts giving no decisive answers to the question of whether melatonin is or is not an effective antiaging agent. Almost all studies are somehow invalid when applying the strict guidelines for long-term testing of chemicals and correct gerontological evaluation of efficacy *(16)*. The most important critical points are (a) few animals are evaluated with no random sampling, recruitment, or other statistical problems associated with the use of improper test cohorts; (b) treatments begun late in life and/or observations stopped voluntarily too early, not extending to the natural death of the last survivor; (c) lack of the necessary autopsy and correct pathomorphological examinations; (d) food and fluid consumption as well as body weight gain are not monitored; (e) no aging markers other than mortality are reported; and (f) most important, but often overlooked, it is not ensured that melatonin

administration really had any effect on circulating or tissue melatonin levels in the treated animals.

Other relevant issues are not easily addressed and some problems cannot be resolved satisfactorily. For instance, adding melatonin to the chow can result in oxidation of the indoleamine within hours. Thus, melatonin is not administered, but rather the oxidation products are tested for their effects on longevity. Moreover, many mouse strains reduce food intake when melatonin is added to the chow. This may lead to false-positive results due to a dietary restriction and enhanced life span by reduced caloric intake. The most popular method is to administer melatonin with the drinking water; often a vehicle is used. Fluid intake has to be measured and the vehicle has to be evaluated for possible effects on longevity; this is almost never reported in the publications available.

In a recent study demonstrating enhanced survival, performance and fitness of aging transgenic mice after chronic melatonin treatment *(45)*, we experienced such problems using non-transgenic control animals treated with vehicle or melatonin, including, but not limited to initial melatonin degradation and formation of toxic oxidation products in the drinking fluid (this problem could be addressed by using covered dark bottles and changing the drinking water frequently); high variability in melatonin intake and very low, with time, declining efficacy in enhancing tissue melatonin levels although 50–100 mg/kg/d of melatonin were provided continously with the drinking water (with exception of the enzyme induction due to long-term melatonin administration, this problem can be partially compensated using a different route of administration such as ip or sc, although these routes may cause pain and continuous stress); high morbidity and totally different appearance of control animals vs melatonin-treated mice, deblinding our samples (with possible introduction of bias) and forcing us to prematurely sacrifice many vehicle-treated old, non-transgenic mice (because of strict animal protection laws, rendering the samples useless for statistical purposes).

Interestingly an effect referred to as the "melatonin methusala syndrome" was observed: thus, melatonin-treated mice finally died without showing any obvious signs of pathology. They retained a luxurious coat with no bald patches, rarely was cancer or other signs of diseases such as osteoporosis present; this condition was frequently present in the control animals. Despite these exciting and interesting observations, the numerous issues mentioned above and many other additional severe experimental problems with the control cohorts have limited the veracity of these data. Nevertheless, the findings were impressive, with at least a 70% increase in 50% and 90% survival, a much better cognitive performance, reduced protein oxidation and nitration, as well as amyloid and disease burden in melatonin-treated animals. Similar results have been obtained in a small sample of male and female Sprague–Dawley rats treated with a similar pharmacological dose. The experiments had to be terminated because of financial constraints (some of the melatonin-treated animals approached 6 yr of age) and ethical concerns (in contrast to the melatonin-treated rats, many old non-melatonin-treated animals suffered from chronic diseases associated with pain such as osteoporosis, cancer, and inflammation of the bald skin patches, which was not acceptable in terms of animal protection).

Because health problems associated with advanced age are an increasing problem, more time and resources should be devoted to study the aging process and its causes. The most promising area of future research is to identify mechanisms leading to the age-related pathologies afflicting many elderly. The focus on this experimental work could guide us in developing a more efficient medicine for people of old age and in the future will hopefully address their important specific health care needs for prevention and protection. Innovative and efficient programs and new animal models as well as advanced test systems to evaluate intervention strategies to enhance the active life span and promote healthy aging are urgently needed in experimental gerontology. Because of their severe limitations as outlined above, the current animal models used in experimental gerontology based primarily on the use of laboratory rodents may not have a bright future; the development of alternate methods with advanced technology and broader perspective are strongly suggested.

Prevention of Age-Associated Oxidative Stress

There are a number of publications that have provided robust findings suggesting that exogenously adminstered melatonin may serve to extend life span in well-established animal models, but even these studies do not allow a definitive final conclusion regarding the effect of melatonin on the aging process. Experiments using invertebrates with few exceptions generally support the hypothesis that melatonin plays an important role in delaying the aging process and its associated pathology; however, evidence for this concept in mammals is less compelling, with only a few publications presenting firm proof that melatonin is a potent antiaging agent. There is no ambiguity about the consequences of melatonin deficiency and melatonin supplementation: melatonin deficiency is always associated with prooxidant effects, whereas melatonin supplementation provides profound antioxidant protection with increased survival and reduced oxidative stress and damage *(14,15,46,47)*.

To preserve health in old age is a primary goal for biomedicine, because increasing longevity is associated with a rise in morbidity and mortality resulting from many age-related pathologies. The difficulties of the search for effective gerontoprotective agents are related to the lack of theoretical background as well as insufficient models to test efficacy and safety of these compounds *(48)*.

Aging is a complex process with multiple mechanisms and causes affected by many genes and thus many biochemi-

cal pathways. This situation poses fundamental challenges to any development of an antiaging medicine to control its pathophysiology *(48)*. The complexity of the aging problem results in difficulties in understanding the basic mechanisms driving the process.

Among the many theories on the mechanisms of aging, one suggests the involvement of oxygen free radicals *(48–51)*. According to this idea, mitochondrial oxidative stress and damage play causative roles in the aging process, a view that is increasingly accepted, although many reviewers have stressed the notion that coincidence does not necessarily prove causality.

The concept linking oxidative stress and damage to the aging process and age-related pathology is primarily based on three major lines of still circumstantial evidence: (a) Reactive intermediates of oxygen reduction are constantly generated at a high rate in mitochondria of all aerobic organisms. (b) Products of oxidative damage accumulate exponentially in senescent organisms under physiological and even more so under pathophysiological conditions. (c) Factors that enhance oxidative stress can greatly increase mortality and morbidity and accelerate the aging process, whereas certain antioxidants can slow the rate of aging and the incidence and severity of age-related pathology.

The free radical theory of aging as first proposed by Harman *(49,50)* was then modified to stress the role of mitochondria as the main source and target of these reactive intermediates. Inspired by earlier observations by Gerschman and associates *(51)* on the similarity between the damaging effects of oxygen and ionizing radiation, Harman *(49)* suggested that the aging process might be driven by the exponential increase in oxidative stress and damage, which, at some point, may become irreversible, resulting in the death of cells and organisms.

If radicals contribute to aging and age-related diseases, this process should be slowed by decreasing their biological effects or by increasing antioxidant defense and repair activities. This effect has been observed not only after administration of indoles such as melatonin, but also after the administration of catalytic antioxidants and nitrones *(48–51)*.

In a many studies, it has been demonstrated that advanced catalytic antioxidant compounds of different chemical nature can indeed significantly increase life span of animals, although these drugs are not always effective in increasing survival as a gerontoprotective agent *(48–51)*. Whereas sometimes only limited effects on longevity or even a reduction of survival after treatment with a nitrone antioxidant has been recorded, the majority of studies have yielded significant and substantial increases of mean and maximum life span in vitro and in vivo after administration of such compounds, a situation very reminiscent of work with melatonin *(48–51)*.

A strong demand thus exists for conclusive studies on aging and antioxidant agents such as indoles and nitrones *(48)*. As broad spectrum antioxidant agents, potent radical scavengers such as melatonin are prime candidates for such testing. Based on a well-established animal model using rotifers of the genus *Philodina,*there is now firm and unequivocal evidence that treatment with amphiphilic antioxidant agents not only prevents age-related oxidative stress and damage, but coincidentally causes significant increases of mean and maximum life span. The advantages of this invertebrate animal model are high-throughput, low-cost, and time-saving experimental protocols owing to fast growth and short generation time. This highly efficient and cost-effective test system allows a rapid screening of drugs for bioactivity, toxicity, and bioavailability in vivo and is superior to rodent models, which are much more labor intensive, expensive, and time consuming.

In these bdelloid rotifers, all physiological parameters indicate that antioxidant treatment with indoles such as melatonin and its kynuramine metabolites or the related compounds indole-3-propionic acid and indole-3-propionamide (M. A. Pappolla et al., unpublished data) as well as amphiphilic antioxidant nitrones (B. Pucci et al., unpublished data) can protect against the degeneration and death associated with senescence and greatly increase rotifer growth, fecundity, and longevity. This rotifer aging model was modified to yield highly consistent results, and a robust culture system allowed for a large-scale analysis of the physiological effects of antioxidative drugs on individually housed organisms of defined and uniform age. The culture conditions also allowed for a rapid screening of bioactivity and toxicity.

The antiaging effects of chronic melatonin treatment at low micromolar concentrations in this rotifer model were robust, reproducible, and impressive and could not be matched by any other antioxidant agents. Amphiphilic advanced antioxidant agents such as melatonin were far superior to other previously tested antioxidant compounds and at least one order of magnitude more potent than any previously documented antiaging strategies such as α-tocopherol *(52)*. Interestingly, not only amphiphilic antioxidant indole and nitrone compounds with high antioxidant acitivity are effective in extending mean and maximum life span, but also the melatonin metabolites AFMK (N^1-acetyl-N^2-formyl-5-methoxykynuramine) and AMK (N^1-acetyl-5-methoxykynuramine). All these compounds completely prevented the pronounced age-dependent accumulation of peroxides in these organisms, an excellent marker for oxidative stress and damage. Coincidentally, size and fecundity of treated rotifers were dramatically increased.

The higher percentage of survival in treated animals was associated with an increase in average body length, average duration of the reproductive period, and a higher number of offspring per rotifer as conclusively demonstrated in this study. These data indicate that oxidative stress is a major determinant of longevity and confirm a significant gerontoprotective activity for compounds with antioxidant activity in single-housed rotifers as previously suggested by Enesco and Verone-Smith *(52)*. Furthermore, because only certain amphiphilic antioxidants such as melatonin

were active, we assume that mitochondria may indeed be the origin of the enhanced radical formation observed during aging of these organisms.

It is interesting to note that higher concentrations of lipophilic antioxidants can be toxic to rotifers, whereas amphiphilic antioxidants such as melatonin are tolerated extremely well, when given in concentrations as high as 1 m*M* and even above up to the solubility limit, three orders of magnitude higher then administered in the survival studies. The unexpected toxicity of lipophilic antioxidants at higher concentrations may explain the failure of some studies using a nitrone as an antiaging drug and confirm that higher concentrations of such drugs decrease, rather than increase, mean life span. During these studies it was consistently observed that only the very amphiphilic antioxidant agents are potent protectants with high bioactivity.

Compounds lacking the ability to penetrate intracellular compartments with limited bioavailability were completely ineffective. Thus, high bioavailability enabled by an enhanced amphiphilicity *(53)* seems to a key issue in determining bioactivity in rotifers. Antioxidant protection against highly reactive free radicals is only successful if on-site protection is possible *(5)*. Highly reactive free radicals must be scavenged at the site of their production *(5)*. Immediate detoxification of these reactive intermediates is a precondition for efficacy of antioxidant drugs explaining why most conventional radical scavengers and highly hydrophilic antioxidants show no or little protection in vivo as in the rotifer model despite their high capacity to detoxify reactive intermediates in in vitro experiments.

Melatonin is an antioxidant that crosses all biological barriers with ease *(54,55)* and the indoleamine can therefore penetrate all intracellular compartments including mitochondria. In contrast to many other antioxidants including vitamins C and E as well as glutathione, melatonin is an amphiphilic molecule that can target all biological sites of radical generation and toxicity and is potent in providing ubiquitous on-site protection against oxidative stress and damage. Melatonin is both lipophilic and hydrophilic giving it a high and well-balanced degree of amphiphilicity which ensures that the compound exhibits a high bioavailability and distribution within the organism *(54,55)*. The indoleamine can diffuse widely into all organs and cell compartments, thus providing immediate on-site protection against radical-mediated damage to biomolecules.

The age-dependent increase in the formation of these reactive intermediates with devastating effects on numerous biomolecules can be ameliorated with melatonin treatment, because this indoleamine catalyzes one-electron-transfer reactions to reduce and detoxify endogenous radicals wherever they are formed within the organism *(30)*. This unique activity is of great advantage and significantly contributes to the superiority of melatonin and similar amphiphilic compounds of high bioavailability over other conventional antioxidant agents in protection and prevention of oxidative stress and damage caused by free radicals.

Melatonin as Mitochondrial Medicine in Senescence

Mitochondria may be considered a primary target of melatonin and its metabolites. Recently, the accumulating evidence that indoleamines and kynuramines may act as mitochondrial metabolism modifiers with physiological and pharmacological effects far exceeding the direct antioxidant effects have been summarized and reviewed *(10,30, 56)*. A new role for biogenic amines as modulators of electron transport and energy metabolism in preventing electron leakage and collapse or the proton potential has been proposed *(30)*. Recent independent evidence from several laboratories indicates that melatonin-derived kynuramines could act as very potent mitochondrial metabolism modifiers *(57)*. Mitochondria could even be the primary target of these endogenous agents and multiple mechanisms have been tentatively identified by which the function of these organelles could be supported and maintained even under adverse conditions.

The kynuric metabolites of indole compounds such as melatonin have potent antiamyloidogenic and antiinflammatory effects sometimes even exceeding those of their parent compounds. It has long been known that AMK, a brain metabolite of melatonin, is a potent inhibitor of prostaglandin synthesis *(58)*. This kynuric as well as other anthranilic compounds derived from indole antioxidants such as indole-3-propionic acid *(59)* are selective cyclooxygenase-2 inhibitors. They markedly reduce the formation of inflammatory lipid mediators, which may have detrimental effects on mitochondrial function and integrity. Melatonin may not only be loaded to and metabolized in mitochondria, but may also act as a prodrug in these powerhouses of the cells to stabilize the membrane domains carrying the specific electron-donor–acceptor complexes that bind electron carriers and thereby enable energy metabolism.

Interestingly, an observation dating back more two decades, showing that melatonin, AMK, and other endogenous tryptophan metabolites are potent modulators of diazepine binding sites *(60)*, may not only explain some of the neuroinhibitory effects of these antiexcitotoxic compounds (R. Schwarcz et al., unpublished findings) but may also have very profound implications for mitochondrial physiology and pathophysiology. About a decade ago, Menendez-Pelaez et al. *(54,55)* reported that melatonin penetrates all intracellular compartments and may intercalate to DNA. They described a primarily nuclear localization of the indoleamine in many different mammalian tissues *(54,55)*, but also observed a strong mitochondrial accumulation of the indoleamine (A. Menendez-Pelaez, unpublished findings). Melatonin has a very high affinity to a specific protein binding site in these organelles. Some evidence indicates

that the mitochondrial peripheral benzodiazepine receptor (PBR), which is considered to be involved in apoptosis induction *(61)*, may be this specific target of melatonin and its metabolite AMK. This may explain the direct inhibition of mitochondrial permeability transition and the potent anti-apoptotic effects of melatonin and related indole and kynuric compounds. Interestingly, amphiphilic nitrones are also potent modulators of this mitochondrial binding site and membrane peroxidation.

Melatonin and metabolites may be potent mitochondrial medicine and correct dysfunction by acting as electron interceptors increasing oxygen and energy metabolism efficacy *(56,57)*. Because many age-related diseases are somehow also based on mitochondrial dysfunction, energy deprivation, and oxidative stress, agents such as melatonin and its metabolites may be the first compounds to allow for a real causative treatment of these pathologies *(4,10,12,30,34,56, 57)*. This area of research is one of the most promising and rapidly expanding fields in biomedicine, which should have our full attention. The focus on mitochondria and their role in aging and its pathology may be the best opportunity to make fast progress in finding intervention strategies that address the mechanisms leading to increased morbidity and mortality with advanced age.

Melatonin preserves mitochondrial function and restores the disrupted oxygen and energy metabolism to normal in old animals *(12)*. The aging process leads to mitochondrial dysfunction with electron leakage, collapse of the proton potential, and a remarkable loss in respiratory control *(12)*. Melatonin administration prevents these highly detrimental effects demonstrated in old senescence-accelerated mice *(12)* and reinstitutes mitochondrial respiratory activity and oxidative phosphorylation to normal *(12)*. The efficacy of electron transport and oxygen utilization is improved and activity of the respiratory chain is increased after long-term melatonin adminstration.

Young, healthy animals and those that are senescence-resistant do not show the age-related deterioration in mitochondrial function and energy metabolism efficacy demonstrated in senescent-accelerated mice *(12)*. Therefore, there is good reason to assume that this beneficial effect of melatonin is highly specific and chronic administration of this indoleamine may prevent premature aging in mammals.

New animal models of mitochondrial dysfunction in the context of aging and degenerative diseases to find new targets for disease prevention and treatment are currently being used. Advanced protective agents such as melatonin may improve oxygen utilization and reduce the damage and degeneration induced by age-dependent pathologies of mitochondrial origin.

Melatonin is a natural compound of low toxicity and, as such, a prime candidate for protection studies testing amphiphilic antioxidants in humans. After a careful evaluation of the safety of such agents, the potential of these mitochondrial metabolism modifiers with potent antioxidant activity in slowing the aging process and targeting disease progression should be considered.

Concluding Remarks

It is generally accepted that normal and premature aging and many associated pathologies and diseases lead to internal desynchronization and dysdifferentiation as consequences of mutation and degeneration. Knowledge of the basic biology of aging and a better understanding of the mechanisms driving this developmental process and determining its rate will be invaluable to meet the increasing demand posed by the large percentage and absolute number of elderly in our society.

Adding more life to our years rather than adding more years to our live should be the primary goal of experimental gerontological research and geriatric care. There is a huge demand and sometimes even desperate need for innovative concepts of effective and safe intervention strategies to combat premature aging and age-related diseases to ensure a healthy and happy life well into advanced age. If a delay in the onset of age-related diseases and slow down of their progression could be achieved, the most ambitious aim of improving the well-being of the growing elderly population in our societies would be reached.

Melatonin supplementation is a prime candidate for a strategy based on prevention and protection that may enhance both health and longevity. Factors that could significantly slow the functional decline of organisms with age and their ability to cope with every day life and its challenges are molecules such as melatonin *(62)*, a well-balanced low-caloric diet, daily exercise, and ample social support. By markedly reducing overall disease prevalence, supplementation with antioxidants may ameliorate detrimental age-dependent changes and thereby greatly benefit old people.

Well-conducted clinical studies addressing the safety issues of chronic melatonin administration are urgently needed. A large-scale evaluation of the effects of voluntary melatonin supplementation by the elderly is overdue and would be of great help in analyzing benefits and possible pitfalls of melatonin therapy. The primary goal of future medicine should be to maintain health in old age, and the focus of our efforts should concentrate on increasing the quality of life rather than on extending life span. Melatonin may be uniquely suitable to achieve such an ambitious aim.

The age-associated exponential increase in oxidative stress and damage, which is even further enhanced in many diseases, can be efficiently antagonized by melatonin treatment. Neurodegenerative diseases and their underlying age-dependent pathology are excellent targets for the antioxidant intervention strategies using melatonin. Experimental work using transgenic animal models of such diseases have now provided firm and consistent evidence that a delay

of disease onset and progression can be achieved by administering pharmacological doses of the indoleamine *(1,2,45)*.

Because melatonin was used safely in humans under controlled conditions, even after chronic administration of higher concentrations of the indoleamine *(63)*, clinical studies on melatonin should be in the focus of future aging research and are strongly encouraged. Humans afflicted by these debilitating and devastating diseases of old age may greatly benefit from the antioxidant effects of melatonin.

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