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## The key role of growth hormone — insulin — IGF-1 signaling in aging and cancer

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### Abstract

Studies in mammals have led to the suggestion that hyperglycemia and hyperinsulinemia are important factors in aging. GH/Insulin/insulin-like growth factor 1 (IGF-1) signaling molecules that have been linked to longevity include daf-2 and InR and their homologues in mammals, and inactivation of the corresponding genes increases lifespan in nematodes, fruit flies and mice. The life-prolonging effects of caloric restriction are likely related to decreasing IGF-1 levels. Evidence has emerged that antidiabetic drugs are promising candidates for both lifespan extension and prevention of cancer. Thus, antidiabetic drugs postpone spontaneous carcinogenesis in mice and rats, as well as chemical and radiation carcinogenesis in mice, rats and hamsters. Furthermore, metformin seems to decrease the risk for cancer in diabetic patients.

### Keywords

Growth hormone; IGF-1; Antidiabetic biguanides; Aging; Cancer prevention

## 1. Introduction

Interest in the relationship of secretions of the endocrine glands to aging predates modern science and medicine and includes early, and by current standards, naïve attempts of rejuvenation. When methods for measuring hormones in blood were developed, many investigators explored age-related changes in hormone levels as biological markers and also as possible mechanisms of aging.

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A new chapter in the understanding of the relationship of hormones and aging was opened by the demonstration that genetic alterations of hormonal signaling in a microscopic worm, *Caenorhabditis elegans*, can have a profound impact on longevity [1,2]. These exciting findings were soon followed by evidence that *C. elegans* genes coding for various components of the signaling pathway that controls aging are homologous to mammalian genes that control transmission of insulin and insulin-like growth factor (IGF) signals [3,4]. Further work established that the insulin/IGF-like signaling (IIS) pathway controls aging in worms, insects and mammals, and those homologies of the genes involved also extend to unicellular yeast [5-7]. In each of these organisms, genetic down-regulation or interruption of this signaling pathway can lead to major extension of longevity.

In female mice, lifespan can be increased by heterozygosity for the deletion of IGF-1 receptors [8] by increasing local (tissue) levels of free, bioavailable IGF-1 [9], by deleting insulin receptors selectively in the adipose tissue [10], and by deleting insulin/IGF-1 signaling intermediates [11,12]. Some of these genetic interventions also extend life in males [9,12]. Moreover, robust extension of longevity in both sexes was detected in mice lacking growth hormone (GH) or GH receptors [13-15] in which circulating levels of IGF-1 are profoundly suppressed, insulin levels are reduced and insulin sensitivity is enhanced [16].

Phenotypic characteristics of long-lived GH-related mouse mutants include reduced incidence and delayed onset of cancer [17-19], prolonged maintenance of youthful levels of cognitive function [20,21], delayed immune aging [14] and marked extension of “healthspan,” i.e., length of life free of disease and functional deficits [14,16,19].

In addition to documenting the importance of GH and GH-dependent alterations in IIS in mammalian aging, studies in long-lived mutant mice indicated that physiological processes related to growth and metabolism involve significant “costs” in relation to aging and longevity. This leads to an important conclusion that interventions affecting these processes could slow aging and offer protection from age-related disease. In the following sections of this article, we will present evidence that extension of longevity in GH-related mutants is associated with partial protection from cancer; discuss mechanisms linking reduced GH and IGF-1 signaling with extension of healthspan and lifespan; and identify those findings in mutant mice that apply to the control of human aging.

The effects of factors or drugs that increase lifespan (geroprotectors) on spontaneous tumor development may provide important clues to the interactions of aging and carcinogenesis. A number of substances were shown to extend lifespan [22-25]. However, these pharmacological interventions in the aging process were sometimes associated with unfavorable side effects. Data comparison on the mechanisms of action of geroprotectors with their influence on the development of spontaneous and experimentally induced tumors deepens our understanding of the interactions between two fundamental biological processes — aging and carcinogenesis [22,26,27]. The main goal of this review is critical evaluation of available data on the effects of antidiabetic drugs on aging in experimental animals and on the perspective of practical uses of these drugs for cancer prevention and healthy aging enhancement in humans.

## 2. Effects of calorie restriction

Calorie restriction (CR) is the only known intervention in mammals that has been consistently shown to increase lifespan, reduce incidence and retard the onset of age-related diseases, including cancer and diabetes. CR has also been shown to increase resistance to stress and toxicity, and maintain youthful levels of function and vitality in laboratory mammals at advanced chronological age [25,28-30]. Studies in CR rhesus monkeys have produced physiological responses strikingly similar to those observed in rodents and delayed the onset of age-related diseases [31-33], but effects on longevity were not consistent [32,33]. Colman and her colleagues [32] reported that monkeys subjected to CR lived significantly longer than control animals fed *ad libitum* (AL) if deaths due to accidents and other causes unrelated to aging were censored from the data. A recent report from another group studying effects of CR in rhesus monkeys reporting no impact on longevity [33], although health of the animals improved, resembling the earlier reports [31,32]. Differences in the feeding protocol of the control (AL) group, origin of the animals and diet composition between the two studies [32,33] undoubtedly contributed to differences between the results. Nevertheless, the question of whether CR can extend longevity in non-human primates remains to be conclusively answered.

Humans voluntarily practicing CR for extended periods of time exhibit many physiological characteristics expected from the studies of CR in rodents, including impressive improvements in risk factors for cardiovascular disease [34,35], but impact of this lifestyle modification on longevity is unknown. Studies of the population of Okinawa, Japan, which until recently had habitually low caloric intake, long life expectancy and exceptionally high frequency of centenarians, indicate that CR can extend human longevity [36]. The questions of whether CR effects observed in experimental animals can be extrapolated to the human and whether CR should be expected to extend human life are hotly debated [37-42]. Conclusively answering these questions is complicated by the logistic and ethical difficulties of conducting well-controlled, long-term studies in the human and by the fact that the mechanisms linking CR with extended longevity in any species are not completely understood. In mammals, multiple interesting mechanisms appear to be involved with a prominent role of metabolic adaptations, hormonal changes and hormetic mechanisms (i.e. benefits stemming from repeated or chronic exposure to the stress of hunger) [28,29,42,43].

The crucial events in the action of CR include reduction in the levels of insulin and insulin-like growth factor-1 (IGF-1) and increases in insulin sensitivity in rodents [36] as well as monkeys [32]. In *Caenorhabditis elegans* and *Drosophila melanogaster*, mutations of genes operating in the signal transduction from insulin receptor to transcription factor *daf-16/dFOXO* (*age-1*, *daf-2*, *InR*, etc.) are strongly associated with longevity [34,43,44]. Whole-genome analysis of gene expression during aging of nematode worm *C. elegans* provided new evidence on the role of insulin homologue genes and *SIR2* homologues in longevity by interacting with the *daf-2/age-1* insulin-like signaling pathway and regulating downstream targets [45].

Activity of *daf-16* counteracts both aging and tumor-like pathology in *C. elegans*. These two processes are physiologically linked because tumor rates invariably rise as an animal ages.

Pinkston-Gosse and Kenyon [46] have identified 29 *daf-16*/FOXO-regulated genes that act in the insulin/IGF-1 pathway to influence tumor growth in *C. elegans*. Some of these genes are required for the effect of *daf-2* mutations on cell death, but none appear to be required for its effect on cell division. Because both increased apoptosis and reduced cell proliferation contribute to the tumor-protective effects of *daf-2* mutations [47], these downstream genes are likely to act in a cumulative fashion to influence tumor growth, much as downstream targets of *daf-16* appear to act cumulatively to influence lifespan [48]. It was shown that almost half of the genes that affect tumor growth also affect the lifespan of animals that do not have tumors, indicating that the ability of the insulin/IGF-1 pathway to couple extended longevity and tumor resistance extends downstream of DAF-16 [46].

*Daf-2* and Drosophila insulin receptors (*InR*) are structural homologues of tyrosine kinase receptors in vertebrates that include the insulin receptor and the IGF-1 receptor. It was shown that, in vertebrates, the insulin receptor regulates energy metabolism whereas the IGF-1 receptor promotes growth. In recent years, a series of elegant experiments in rodents, in which various key elements of the insulin/IGF-1 signaling pathway were genetically modified, provided evidence of this system's involvement in the control of mammalian aging and longevity [7]. It showed that less insulin receptor substrate-2 (*Irs2*) signaling throughout the body or just in the brain extended lifespan up to 18% in mice [11]. Kapeller et al. [49] showed that partial inactivation of brain IGF receptors (IGF-1R) in embryonic brain selectively inhibited GH and IGF-1 pathways after birth. This causes growth retardation, smaller adult size and metabolic alterations, and it leads to delayed mortality and longer mean lifespan. The mean lifespan in *bIGF1RKO*<sup>+/-</sup> mice increased by 9.3% ( $p < 0.05$ ) as compared with control mice, and tumor incidence declined from 53% to 44% ( $p > 0.05$ ).

Selman et al. [50] measured the lifespan of mice lacking either insulin receptor substrate (IRS) 1 or 2, the major intracellular effectors of the insulin/IGF-1 signaling (IIS) receptors. It was observed that female *Irs1*<sup>-/-</sup> mice are long-lived. Furthermore, they displayed resistance to a range of age-sensitive markers of aging including skin, bone, immune and motor dysfunction. These improvements in health were seen despite mild, lifelong insulin resistance. Thus, enhanced insulin sensitivity is not a prerequisite for lifespan extension. *Irs1*<sup>-/-</sup> female mice also displayed normal anterior pituitary function, distinguishing them from long-living somatotrophic axis mutants. In contrast, *Irs2*<sup>-/-</sup> mice were short-lived, whereas *Irs1*<sup>+/-</sup> and *Irs2*<sup>+/-</sup> mice of both sexes showed normal lifespans.

### 3. Mechanisms linking reduced GH signaling with extended longevity

Direct and indirect actions of GH influence a multitude of functions in virtually every tissue. Consequently, suppression or elimination of GH action in GH-deficient and GH receptor deleted mice leads to numerous effects, including alterations in processes known or suspected to influence aging and longevity. For example, in the absence of stimulatory influence of GH on hepatic IGF-1 gene expression, circulating levels of IGF-1 are dramatically reduced. This provides a plausible explanation for cancer incidence being reduced in Ames dwarf, Snell dwarf and GHRKO mice [17-19]. Key characteristics of mutant mice most frequently used to study the impact of GH on aging and longevity are listed in Table 1.

In addition to causing profound suppression of circulating IGF-1 levels, absence of GH signals is associated with reduced activity of the mammalian target of rapamycin (mTOR) signaling pathway [51,52]. This pathway influences growth by stimulating translation, and it is well documented that it is involved in the control of aging in simpler organisms [53]. There is also considerable evidence that it plays a similar role in mammals. Mouse longevity was extended by treatment with rapamycin, an inhibitor of mTOR [24,54,55], and by deletion of S6 kinase1 (S6K1), a target of mTOR that mediates some of its actions [56]. The well-documented role of mTOR in the stimulation of growth suggests that reduced mTOR activity in GH-deficient, GH-resistant and rapamycin-treated mice importantly contributes to these animals' protection from cancer.

Dermal fibroblasts derived from long-lived GH-deficient and GH-resistant mice exhibit enhanced resistance to multiple forms of oxidative, metabolic or cytotoxic stress [57]. Treatment of Ames dwarf mice with GH prior to isolation of fibroblasts suppresses resistance to many of these stresses, i.e., “rescues” the normal (wild type) phenotype [58]. Increased stress resistance of cells derived from long-lived, GH-related mutants likely reflects improved antioxidant defenses [59] as well as different activation of stress-responsible genes and pathways [60]. Association of cellular stress resistance with extended longevity was described in numerous studies of simpler organisms [61,62] and in a study of cells derived from humans genetically predisposed to longevity in comparison to their partners [63]. The importance of these associations is emphasized by the evidence that Ames dwarf mice survive longer than normal animals from the same strain when they are treated with Paraquat, one of the toxic agents to which Ames dwarf fibroblasts were previously shown to be more resistant in culture [64].

Absence of GH or its receptors removes the well-documented anti-insulinemic effect of GH and thus leads to enhanced insulin sensitivity. Combined with the absence of stimulatory effects of GH and IGF-1 on the development and function of insulin-producing cells, enhanced insulin sensitivity leads to a reduction of insulin levels in the circulation. This is particularly pronounced in GHRKO mice. It deserves emphasis that characteristics of insulin signaling in long-lived GH-related mutants: reduced insulin levels and enhanced insulin sensitivity are opposite of the endocrine features of obesity and endocrine syndrome.

In GH-resistant GHRKO mice, enhanced whole animal insulin sensitivity is associated with increased content and activation of insulin receptors in the liver and with reduced inhibitory (Serine 307) phosphorylation of insulin receptor substrate 1 (IRS1) in the skeletal muscles [52]. The latter effect likely reflects reduced activation of JNK1 and mTOR, factors known to promote insulin resistance by phosphorylating this or homologous sites of the IRS1 molecule [52,65].

Other metabolic adaptations believed to link reduced GH signaling with extended longevity include enhanced activity of an important nutrient sensor, AMP-activated kinase (AMPK) [66], increased  $\beta$  oxidation of fatty acids [67] and increased levels of a key regulator of mitochondrial biogenesis, PPAR $\gamma$  co-activator 1  $\alpha$  (PGC1 $\alpha$ ) [66]. These adaptations provide a likely explanation for key features of energy metabolism in long-lived GH-related mutants: an increase in oxygen consumption (VO<sub>2</sub>) and a reduction in respiratory quotient

(RQ) [67]. Low values of RQ indicate an increased reliance on fats, as opposed to carbohydrates, as metabolic fuel. In GHRKO mice,  $VO_2$  [expressed per gram (g) of body weight or per g of lean body mass determined by DEXA] increases during both dark, active and light, quiescent periods of the day. This increase is evident in both presence and absence of food and is detected in animals that had been calorically restricted or fasted every other day (Westbrook & Bartke, unpublished). Interestingly, at a thermoneutral temperature of 30°C,  $VO_2$  of GHRKO and normal mice does not differ. This indicates that increased  $VO_2$  of GHRKO mice at a standard animal room temperature (22-24°C) reflects an increased energy demand for thermoregulation needed to compensate for greater heat loss in these diminutive animals. Apparently, the impact of increased surface to mass ratio on radiation heat loss is not compensated by increased accumulation of subcutaneous adipose tissue in these animals.

Although many mechanisms are undoubtedly responsible for extended longevity of GH-related mouse mutants, we believe that many characteristics of these animals, including slower aging, are directly or indirectly related to altered secretion of pro- and anti-inflammatory cytokines by their adipose tissue. There is increasing evidence that, in the absence of GH or GH receptors, adipose tissue secretes more anti-inflammatory adiponectin and less pro-inflammatory products including interleukin 6 (IL6) and tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) [68]. It is also well-documented that age-related changes in body composition and perhaps aging per se lead to an increase in inflammation [69]. This increase along with life-long “inflammatory load” are believed to be important drivers of aging [69,70], a major risk factor for cancer and other age-related diseases [71], and a predictor of longevity [70].

Studies involving surgical removal of intraabdominal (epididymal and perinephric) fat depots from normal and GHRKO mice provided evidence that adiponectin secretion by these depots of white adipose tissue is responsible for the increased circulating adiponectin levels in these animals [68]. Interestingly, removal of the major depots of intraabdominal fat had the opposite effect on insulin sensitivity in normal and GHRKO mice. While in normal mice this intervention caused the expected improvements in insulin and glucose tolerance, in GHRKO animals it led to deterioration of these two measures of insulin sensitivity [68]. Apparently, adiponectin derived from the perigonadal and/or perinephric fat is largely responsible for the enhanced insulin sensitivity of GHRKO mice. Moreover, elevated levels of adiponectin in these animals likely contribute to or perhaps account for the increases in AMPK activation and  $VO_2$ , which were mentioned earlier in this section.

Remarkable extension of longevity in mice lacking GH or its receptors is associated with equally impressive extensions of healthspan, as evidenced by delayed and/or reduced appearance of age-related diseases including cancer, osteoarthritis and cataracts [reviewed in 16], and improved maintenance of cognitive, immune and neuromuscular functions [14,20,21; Arum & Bartke, unpublished] and apparently also reproductive competence [72]. Intriguingly, these animals are also protected from the age-related depletion of pluripotent, very small embryonic like stem cells (VSELs) in the bone marrow [73]. This could be viewed as yet another marker of delayed aging in these animals and also as another candidate mechanism of extended healthspan and lifespan [73].



#### 4. Implications of findings in long-lived endocrine mutants

Findings in GHRKO, Ames and Snell dwarfs and other GH-related mouse mutants provided evidence that absence of the actions of a hormone can produce major benefits in terms of health and life expectancy. This inescapably leads to a somewhat counterintuitive conclusion that the normal levels and physiological actions of GH are not optimal for disease-free survival or longevity. Because GH is the key determinant of circulating levels of IGF-1 and increases insulin levels as a secondary consequence of its anti-insulinemic actions, conclusions concerning GH and normal aging extend to the roles of IGF-1 and insulin in this process. In support of the implied negative impact of these hormones on aging, excessive levels of GH and IGF-1 in acromegalic humans and transgenic mice, as well as increased levels of IGF-1 and insulin in obesity, are associated with increased risk of age-related disease and reduced life expectancy [74,75]. Moreover, alterations in many characteristics of giant transgenic mice overexpressing GH resemble symptoms of accelerated aging [76].

It is important to emphasize that the negative association of somatotropic (GH, IGF-1) signaling and longevity discovered in mutant, gene knockout and transgenic mice also applies to genetically normal mice, as well as to other species. Growth rate and adult body size, key markers of GH and IGF-1 actions, and plasma IGF-1 levels are negatively correlated with lifespan in comparisons of mice from different stocks or inbred strains, as well as individual animals from a genetically heterogeneous population [77-79]. These relationships also extend to other species including rats, domestic dogs and apparently horses [80-82].

However, even with this evidence it may be difficult to envisage how physiological actions of hormones within normal ranges of their concentration can be detrimental for something as fundamental as longevity. The most likely explanation for this paradox can be found in the concept of antagonistic pleiotropy, which emerged from analysis of the genetic control of aging [83]. Antagonistic pleiotropy explains how genes that exert positive effects on growth, development, sexual maturation and fertility — key elements of the evolutionary fitness, i.e., probability to produce offspring — will be selected for and persists in the population even if the genes have detrimental effects during later stages of life history. Along with other anabolic hormones, GH and its actions fit well with this concept of co-existence of early beneficial and late detrimental effects. In juveniles, GH promotes somatic growth, sexual maturation and various aspects of gonadal function; in adults it increases the risk of cancer, insulin resistance and diabetes. Somatotropic signaling would not have been suppressed or eliminated by natural selection on the basis of these detrimental effects because the force of the natural selection declines with age and is presumably negligible during the post-reproductive period. Also, in the sense of evolutionary fitness promotion of growth, development and fertility far outweigh the risks of late life disease. In this conceptual framework, it is understandable how physiological actions of GH, IGF-1 and insulin could include significant “costs” in terms of aging, cancer and life expectancy. It is also important to mention that long survival can be facilitated by re-directing energy and other resources from growth and reproduction to maintenance, repair and stress resistance, as well as other trade-offs.

## 5. Growth hormone, IGF-1, insulin and human aging

The role of somatotrophic signaling in the control of human aging and longevity is poorly understood, controversial and clouded by commercial interests in promoting GH, as well as various, largely unproven GH-related products, as anti-aging medication. There is a surprising paucity of data concerning aging of individuals with syndromes of GH deficiency or resistance, and longevity of the affected individuals was reported to be reduced [84], normal (possibly reflecting a combination of increased early mortality and reduced late mortality) [85] or perhaps extended [86]. Moreover, it is well documented that some Laron dwarfs, as well as some hypopituitary individuals with mutation of Prop 1, the same gene that is mutated in Ames dwarf mice, can reach an advanced age [86,87]. Interpretation of these findings is further complicated by many of the affected individuals receiving hormone replacement therapy and by the evidence that dwarfism can be associated with increased mortality from causes unrelated to aging, such as alcohol abuse, accidents and seizures [85].

In contrast to limited and somewhat inconsistent data concerning longevity, there is clear evidence that Laron syndrome (GH resistance) provides impressive protection from cancer and diabetes [85,88]. Moreover, GH-deficient individuals were shown to be protected from atherosclerosis in spite of increased adiposity and unfavorable lipid profiles [88].

In several studies, genetic variants with reduced somatotrophic signaling were more common in exceptionally long-lived people [89,90]. Moreover, variants in the gene coding for the transcription factor FOXO, which is under IIS control, are associated with longevity in a number of unrelated human populations [91-94].

Intriguingly, several phenotypic characteristics of long-lived mutant mice, including reduced insulin and glucose levels, increased levels of adiponectin and improved insulin sensitivity, have been detected in centenarians [95-98] and healthy, middle-aged individuals from long-lived families [99,100]. It also deserves emphasis that increased risk of age-related diseases, reduced life expectancy and other symptoms of accelerated aging are associated with GH excess in acromegaly and with dysregulation of metabolic control by insulin in diabetics (details and references earlier in this article).

It was shown that the incidence of mutations in insulin regulatory region (IRE) of APO C-III T-455 C directly correlates with longevity in humans [101]. This is the first evidence that a mutation located downstream to *daf-16* in the insulin signal transduction system is associated with longevity [101]. It is worth noting that centenarians display a lower degree of insulin resistance and a lower degree of oxidative stress as compared with elderly persons less than 90 years old [95,102]. The authors suggest that centenarians may have been selected for appropriate insulin regulation as well as for the appropriate regulation of tyrosine hydroxylase (TH) gene, whose product is rate-limiting in the synthesis of catecholamines, stress-response mediators. It was shown that catecholamines may increase free-radical production through induction of the metabolic rate and auto oxidation in diabetic animals [103]. A study on the aging parameters of young (up to 39 years old) and old (over the age of 70) individuals with similar IGF-1 serum levels provided evidence of this peptide's important role in life expectancy [104]. Roth et al. [105] analyzed data from



the Baltimore Longitudinal Study of Aging and reported that survival was greater in men who maintained a lower insulin level. In women, genetic variations causing reduced insulin/IGF-1 signaling pathway activation is beneficial for old-age survival [89].

Hyperglycemia is an important aging factor involved in the generation of advanced glycation end products (AGEs) [106-108]. Untreated diabetics with elevated glucose levels suffer many manifestations of accelerated aging, such as impaired wound healing, cataracts, vascular and microvascular damage [109]. The accumulation of the AGE, pentosidine, is accelerated in diabetics and has been suggested as a reliable biomarker of aging [106]. The action of insulin provides the major modulator of glucose storage and utilization. It is important to stress that hyperinsulinemia is also an important factor in the development of cancer [109-114]. Although understanding the role of GH and IIS in the control of human aging is incomplete and somewhat controversial, available data indicate that dietary prevention of excessive IGF-1 and insulin secretion and using diet and exercise to enhance insulin sensitivity may represent the most hopeful approaches to cancer prevention and extending human healthspan and lifespan.

## 6. Pharmacological effects on GH/Insulin/IGF-1 signaling pathway and longevity

The concept of CR mimetics is being intensively explored [35,115-118]. CR mimetics are interventions that produce physiological and anti-aging effects similar to CR without limiting caloric intake. Reviewing the available data on the benefits and adverse effects of CR and genetic modifications, Longo and Finch [119] suggested three categories of drugs that may have potential to prevent or postpone age-related diseases and extend lifespan: drugs that (1) simulate dwarf mutations by decreasing pituitary production of growth hormone (GH); (2) prevent IGF-1 release from the liver; or (3) decrease IGF-1 signaling by the action on either extracellular or intracellular targets.

The antidiabetic drugs, phenformin (1-phenylethylbiguanide), buformin (1-butylbiguanide hydro-chloride) and metformin (N,N-dimethylbiguanide) were observed to reduce hyperglycemia; improve glucose utilization; reduce free fatty acid utilization, gluconeogenesis, serum lipids, insulin and IGF-1; reduce body weight; and decrease metabolic immunodepression both in humans and rodents [109,110,120,121]. Currently, phenformin is not used in clinical practice due to its side effects (mainly lactic acidosis) observed in patients with non-compensated diabetes. It is worthy of note that during more than a 10-year-long experience of phenformin administration to patients without advanced diabetes, Dilman and Berstein [122-127] observed no cases of lactic acidosis or any other side effects. We believe that results analysis of long-term administration of this drug as well as other antidiabetic biguanides (buformin and metformin) to non-diabetic animals is important for understanding the links between insulin and longevity and between insulin and cancer.

Forty years ago, it was suggested to use biguanide antidiabetic drugs as a potential anti-aging treatment [109,128]. There are data on the effects of the biguanides on lifespan in worms, mice and rats. Buformin was supplemented to nutrient medium in various

concentrations (from 1.0 to 0.00001 mg/ml) during the larval stage and over the lifespan of *C. elegans*. The drug given at a concentration of 0.1 mg/ml increased the mean lifespan of the worms by 23.4% ( $p < 0.05$ ) and the maximum lifespan by 26.1% as compared to the controls [129]. Metformin supplementation (50 mM dose) was shown to increase the mean, but not maximum, lifespan of *C. elegans*, although 10 or 100 mM doses showed no significant lifespan benefit [130]. These authors have shown that metformin prolongs nematode healthspan, slows lipofuscin accumulation, extends mean lifespan and prolongs youthful locomotor ability in a dose-dependent manner [130]. Genetic data suggest the metformin acts through a mechanism similar to that operative in eating-impaired CR mutants, but independent of insulin signaling pathway. Energy sensor AMPK and AMPK-activating kinase LKB1, which are activated in mammals by metformin treatment [131,132], are essential for health benefits in *C. elegans*, suggesting that metformin engages a metabolic loop conserved across phyla [130]. It was also shown that metformin activated SKN-1/Nrf2, oxidative stress-responsive transcription factor.

The available data on the effect of antidiabetic biguanides on lifespan in mice and rats are summarized in Table 2.

Female C3H/Sn mice were fed a standard diet *ad libitum* and starting at 3.5 months of age were given phenformin 5 times a week orally at a single dose of 2 mg/mouse until a natural death [133]. The treatment with phenformin prolonged the mean lifespan of mice by 21% ( $p < 0.05$ ), the mean lifespan of the last 10% of survivors by 28% and the maximum lifespan by 5.5 months (26%) in comparison with the controls. At the death of the last mice in the control group, 42% of phenformin-treated mice were alive. In this study, food consumption in control and drug-exposed groups was not measured.

Phenformin was given orally in a single dose of 5 mg/rat/day 5 times a week to female outbred LIO rats starting at the age of 3.5 months until a natural death [22,139].

Administration of phenformin failed to influence the mean lifespan in these animals. At the same time, the mean lifespan of the last 10% of survivors increased by 10% ( $p < 0.005$ ), and maximum life span increased by 3 months (10%) in comparison with the controls. The treatment with phenformin slightly decreased the body weight of rats in comparison with the control ( $p > 0.05$ ). The disturbances in the estrus function were observed in 36% of 15-16-month-old rats in the control group and only in 7% of rats in the phenformin-treated group ( $p < 0.05$ ).

Buformin was given to female LIO rats orally in a single dose of 5 mg/rat/day 5 times a week starting at the age of 3.5 months until a natural death [139,140]. The treatment slightly increased mean rat lifespan (by 7%;  $p > 0.05$ ). The mean lifespan of the last 10% of survivors increased by 12% ( $p < 0.05$ ), and the maximum lifespan increased by 2 months (5.5%) as compared with controls. The body weight of rats treated with buformin decreased slightly (5.2 to 9.4%) but statistically significantly ( $p < 0.05$ ) compared with the controls from age 12 months to 20 months ( $p < 0.05$ ). At 16-18 months, 38% of control rats had disturbances in the estrous cycle including persistent estrus, repetitive pseudopregnancies or anestrus. In females treated with buformin, these disturbances were observed only in 9% of

rats ( $p < 0.05$ ). Again, in both of these studies food consumption in the control and drug-exposed groups was not measured.

Long-term administration of metformin (100 mg/kg in drinking water) slightly decreased food consumption but did not change the body weight or temperature; slowed down the age-related rise in blood glucose and triglyceride levels; delayed the age-related decline in estrous cyclicity; prolonged the mean lifespan by 8% ( $p < 0.05$ ); and increased the mean lifespan of the last 10% of survivors by 13.1% and the maximum life span by 1 month in transgenic HER-2/neu mice in comparison with the control animals [134]. The demographic aging rate represented by the estimate of respective Gompertz's parameter decreased 2.26-fold. The reduction of the serum level of cholesterol and beta-lipoproteins was observed in metformin-treated transgenic HER-2/neu female mice [134]. Metformin treatment decreased food consumption in these mice at age 4 and 6 months, suggesting that "voluntary CR" may be the source of metformin effects [25].

The chronic treatment of female outbred, Swiss-derived SHR mice with metformin (100 mg/kg in drinking water) slightly modified the food consumption but decreased the body weight after the age of 2 months ( $p < 0.01$ ). It also increased the mean lifespan of the last 10% of survivors by 20.8% ( $p < 0.01$ ) and maximum lifespan by 2.8 months (10.3%) in comparison with the control SHR mice [136]. The treatment with metformin failed to influence blood estradiol concentration and spontaneous tumor incidence in female SHR mice. In another set of experiments, female SHR mice were given metformin at the same dose starting at age 3, 9 or 15 months [137]. It was observed that metformin decreased body temperature and postponed age-related switching-off of estrous function. Metformin did not affect levels of serum cholesterol, triglycerides, glucose and insulin. Treatment with metformin started at age 3 months increased mean lifespan by 14% and maximum lifespan by 1 month. The treatment started at age 9 months increased mean lifespan by only 6%, whereas the treatment started at age 15 months failed to increase lifespan.

Transgenic mice with Huntington's disease (HD) (the R6/2 line expressing exon 1 of the Huntington protein including ~130 glutamine repeats) were given metformin in drinking water (2 or 5 mg/ml) starting from age 5 weeks [142]. Metformin treatment significantly prolonged (by 20.1%) the survival time of male (but not female) HD mice at the 2 mg/ml dose (~300 mg/kg/day) without affecting fasting blood glucose levels. This dose of the drug also decreased hind limb clasping time in 11-week-old mice. The higher dose of metformin did not prolong lifespan, and neither dose was effective in female HD mice.

In the study of Smith et al. [141], 6-month-old male F344 rats were randomized to one of four diets: control, calorie restricted (CR), metformin (300 mg/kg/day) and pair-fed to metformin. The CR group had significantly reduced food intake and body weight throughout the study. Body weight significantly decreased in the metformin group compared with control during the middle of the study, despite similar weekly food intake. There were no significant differences in the mean lifespan or the mean of the last surviving 10% of each group in the CR group, metformin and pair-fed groups compared with control. However, the aging rate estimate ( $\alpha$  – slope, rate of increase of mortality) of the Gompertz model of the control group alone was significantly different from the three other groups, reflecting the

early deaths in the CR, metformin and pair-fed groups. CR significantly increased lifespan in the 25<sup>th</sup> quantile but not the 50<sup>th</sup>, 75<sup>th</sup> or 90<sup>th</sup> quantile. The groups of rats exposed to metformin or to the pair feeding were not significantly different from controls at any quantile [141]. The authors stressed one limitation of this study — the lack of a robust CR response in terms of extension of maximum lifespan, which has been observed in another CR study using the same strain of rats [143]. The reduced efficacy of CR in this study might provide a partial explanation for the lack of a significant increase in longevity with metformin treatment. In addition to the dampened CR response, metformin treatment did not significantly affect glucose/insulin levels in this study. The metformin concentration utilized in the diet is approximately 10 times that of the highest dose used in human treatments, implying that any increase necessary to observe lifespan benefits is questionable for a human application [141].

Thus, available data provide evidence that antidiabetic drugs can increase rodent survival in some cases (Table 2). This effect was not observed in all experiments and varied depending on strain and species of animals. Female mice and rats have been treated in the majority of these studies. The experiments with males of different strains will be useful for conclusion on geroprotective potential of antidiabetic biguanides.

Metformin is now a widely prescribed medication for treating type-2 diabetes. In addition to improving the metabolic profile of diabetes, increased survival from all-cause mortality has been associated with metformin treatment in both diabetic and cardiovascular disease patients [144,145].

## 7. Antidiabetic biguanides and the endocrine system

Parameters of reproductive function are among the most valuable biomarkers of aging [146]. The treatment with phenformin decreased hypothalamic threshold of the sensitivity to feedback inhibition by estrogens [147,148], which is one of the most important mechanisms regulating age-related decline and eventual cessation of reproductive functions [149-151]. It is noteworthy that another antidiabetic biguanide, metformin, may improve menstrual regularity, leading to spontaneous ovulation, and enhance the induction of ovulation with clomiphene citrate in women with polycystic ovary syndrome [152,153]. The treatment with phenformin also decreased hypothalamic threshold sensitivity to feedback regulation by glucocorticoids and by metabolic stimuli (glucose and insulin) [109]. It was recently shown that elements involved in the insulin/IGF-1 signaling pathway are regulated at the expression and/or functional level in the central nervous system. This regulation may play a role in the brain's insulin resistance [154], in the control of ovarian follicular development and ovulation [155], and in the brain's control of lifespan [156,157]. Antidiabetic biguanides also alleviated age-related metabolic immunodepression [109]. These mechanisms can be involved in the geroprotective effect of biguanides.

Treatment with chromium picolinate, which elevated insulin sensitivity in several tissues, including the hypothalamus, significantly increased the mean lifespan and decreased the development of age-related pathology in rats [158]. It was hypothesized that antidiabetic biguanides — and possibly chromium picolinate — regulate tyrosine hydroxylase and

insulin/IGF-1 signaling pathway genes, both of which are associated with longevity [34,159,160]. It was shown that the polymorphism at TH-INS locus affects non-insulin dependent type-2 diabetes [161], and is associated with hypothalamic obesity [162], polycystic ovary syndrome [163], hypertriglyceridemia and atherosclerosis [164].

## 8. Cancer resistance of long-lived mutants

Studies conducted in the 1950s and '60s provided evidence that hypopituitary Snell dwarf mice are resistant to various carcinogens and that growth of transplanted tumors is inhibited in these animals [165-169]. Snell dwarf mice are homozygous for the loss-of-function mutation at the pituitary factor 1 (Pit1) locus. They lack somatotrophs, lactotrophs and thyrotrophs in the anterior pituitary and as a result are GH, prolactin (PRL) and thyroid-stimulating hormone (TSH)-deficient [169]. More recent histopathological studies at the natural end of life provided evidence for reduced incidence of spontaneously developed tumors in these mutants, as well as Ames dwarf mice and GH receptor-deleted (GHRKO) animals. Ames dwarf mice have endocrine defects identical to those in Snell dwarfs as a result of a mutation in the prophet of Pit1 (Prop1) gene, which controls development and differentiation of the same cell lineages that are affected by Pit1 [170]. Growth hormone receptor-deleted mice are GH resistant [171]. In each of these three mutants, circulating levels of IGF-1 are severely depressed [13,14,16].

Reduced tumorigenesis in Snell dwarf mice was reported by Chen et al. [172] and Vergara et al. [19]. In the latter study, the causes of death were compared in 45 Snell dwarf mice and 45 normal controls. While 87% of control mice died of tumors, only 40% of Snell dwarfs did. Hemangiosarcomas, histocytic sarcomas, fibrosarcomas, lymphomas and mammary adenocarcinomas were detected in both types of mice, but the incidence of the latter two types of tumors appeared reduced in the dwarfs ( $p < 0.06$ ) [19]. Ikeno and his colleagues reported delayed occurrence and reduced incidence of tumors in Ames dwarf and GHRKO mice [17,18]. Both Ames dwarf and their normal siblings developed adenocarcinomas, hepatocellular carcinomas, lymphomas and hemangiomas, but the incidence of fatal adenocarcinoma of the lung was significantly lower in the dwarfs while the percent of animals dying from neoplastic disease was numerically (but not statistically significantly) greater in the normal than in the dwarf mice (95% vs 72%, respectively) [17].

In the study of GHRKO and normal mice, lymphomas and hepatocellular carcinomas were encountered in both groups, while adenocarcinomas were seen only in normal animals. The incidence of adenocarcinomas and lymphomas was significantly lower in GHRKO than in normal mice, and the percentage of animals dying from neoplastic disease was also significantly less in GHRKO animals (42% vs 83%) [18].

## 9. Pharmacological modulation of GH/insulin/IGF-1 signaling pathway in cancer prevention and treatment

There is considerable evidence for anti-tumor effects of antidiabetic biguanides both *in vitro* and *in vivo* [(for review see: 173-175)]. In this paper, we shall not discuss this aspect of the

biguanides action. However, we will briefly analyze the capacity of the biguanides to prevent spontaneous and induced tumorigenesis (Table 3).

### 9.1. Mammary carcinogenesis

Long-term treatment with phenformin significantly inhibited (by 4.0-fold,  $p < 0.01$ ) the incidence of spontaneous mammary adenocarcinomas in female C3H/Sn mice infected with murine mammary tumor virus [139]. The tumor yield curve rise also significantly slowed as a result of the treatment. Metformin given with drinking water inhibited mammary carcinogenesis in HER-2/neu transgenic mice [134,135], but it had no effect on incidence or latency of mammary adenocarcinomas in outbred SHR mice [136,137].

Daily oral administration of phenformin or buformin suppressed 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary tumor development in rats [139,176,177]. Phenformin-treated rats tended to have lower serum insulin levels. Treatment with phenformin normalized glucose tolerance, serum insulin and IGF-1 level in rats exposed to intravenous injections of N-nitrosomethylurea (NMU). It also inhibited mammary carcinogenesis in these animals [179]. Bojkova et al. [180] studied oral metformin's chemopreventive effect in mammary carcinogenesis in female Sprague-Dawley rats. NMU administered in two intraperitoneal doses of 50 mg/kg b.w. between the 43rd-55th postnatal days induced mammary carcinogenesis. Metformin was administered in drinking water (at a concentration of 50  $\mu$ /ml and 500  $\mu$ /ml) 13 days before the first NMU dose until the termination of the experiment. The experiment was terminated 18 weeks after the first NMU dose. Metformin did not significantly alter the tumor growth, although a delay in tumor onset was recorded after a higher metformin dose. Metformin altered metabolic and hormonal variables. Insulinemia decreased after either dose of metformin in comparison with control rats without changes in glycemia. Triacylglycerols concentration decreased in liver and increased in serum when compared to controls. Moreover, the higher dose of metformin attenuated lipid peroxidation in the liver.

In a recent study by Zhu et al. [181], mammary cancer was induced in female Sprague-Dawley rats (50 mg/kg MNU, i.p.), and Metformin was fed alone (AIN93G + 0.05 to 1.0% w/w metformin) or combined with 40% dietary energy restriction. While a dosing regimen of 1.0%/0.25% metformin-reduced palpable mammary carcinoma incidence, multiplicity, and tumor burden and prolonged latency, lower doses of metformin failed to inhibit carcinogenesis despite effects on plasma insulin. In human breast cancer, cell growth inhibition was observed only at high concentrations of metformin. Poor *in vivo* and *in vitro* response to metformin may be the result of pharmacokinetic (OCT-1 expression was low in rat mammary cells; OCT-3 was downregulated in mammary carcinoma) and pharmacodynamic (complex I transcripts were higher in mammary epithelial cells from carcinomas versus uninvolved gland) effects. Combined with dietary energy restriction, metformin protected against new tumor occurrence after release from combined treatment. Flow cytometry indicated the presence of cancer-initiated cells in mammary carcinomas. The authors concluded that, as a single agent, metformin possessed limited cancer inhibitory abilities. However, metformin may be an effective component of multi-agent interventions



that target cancer-initiated cells. There is a clear need to identify the conditions under which metformin is likely to prevent and control breast cancer.

## 9.2. Skin carcinogenesis

One hundred and twenty SHR male mice were randomly divided into 4 groups [182]. Their clean-shaven backs were painted with 0.2 ml of 0.05% acetone solution of benz(a)pyrene (BP) twice a week. Group 1 was in control and received no additional treatment. From beginning to end (6 months), the remaining mice received melatonin 2 mg/l with drinking water at nighttime (group 2), metformin 200 mg/l with drinking water during 24 hrs (group 3), melatonin and metformin as in groups 2 and 3 (group 4). There was a separate group of intact animals. Skin tumor frequency decreased among melatonin/metformin-treated mice (groups 1-4) — 83.3%, 66.7%, 60% and 50%; squamous cell carcinoma — 56.73%, 36.7%, 20% and 20%. Treatment with melatonin and metformin and their combinations was followed by significantly lower tumor multiplicity and smaller size, longer latency period and survival of tumor-bearers. After BP, levels of malonic dialdehyde (MDA) and catalase rose in blood serum while concentrations of the latter in skin tumors were higher than in cutaneous homogenates in intact animals. Melatonin and metformin significantly lowered MDA content of blood serum as compared with group 1. Blood serum catalase fell after their joint administration, whereas it was 2.6 times as high in cutaneous homogenates after metformin. However, it decreased after melatonin [182]. Female SHR mice (n=200) were divided at random into 4 groups, 50 per group [183]. BP solution in acetone was applied to a skin site on the back for 26 weeks. In parallel, the animals received melatonin, metformin or both. Melatonin, metformin and their combination promoted significant reduction of the number and size of skin tumors. In mice receiving no therapy, BP applications increased the concentrations of malonic dialdehyde in the serum and skin tumor tissue in comparison with the concentrations in the sera and skin of intact mice. Melatonin, metformin and their combination normalized LPO level [183].

## 9.3. Soft-tissue carcinogenesis

Vinnitski and Iakumenko [184] have shown that treatment with phenformin increased immunological reactivity and inhibited carcinogenesis induced by subcutaneous administration of 20-methylcholanthrene in BALB/c mice.

In the experiments of Deriabina [185] 120 SHR male mice were randomly divided into 4 groups. All animals were subjected to a single, subcutaneous injection of 2 mg of benz(a)pyrene (BP). Group 1 was in control and received no additional treatment. From beginning to end (6 months), the remaining mice received melatonin 2 mg/l with drinking water at nighttime (group 2), metformin 200 mg/l with drinking water during 24 hrs (group 3), and melatonin and metformin as in groups 2 and 3 (group 4). There was a separate group of intact animals. Soft-tissue tumor (malignant fibrous histiocytomas) developed in melatonin/metformin-treated mice (groups 1-4) - 80%, 60%, 46.7% and 40%. Metformin and melatonin, as well as its combination, increased survival of tumor-bearing mice. The author observed significant inhibitory effect of metformin on mitotic activity in tumor tissue [185].

Treatment with melatonin and metformin and their combinations was followed by significantly lower tumor multiplicity (number of tumors per tumor-bearing animal) and smaller size, longer latency period and survival of tumor-bearers. After BP, levels of malonic dialdehyde (MDA) and catalase rose in blood serum while concentrations of the latter in skin tumors were higher than in cutaneous homogenates in intact animals. Melatonin and metformin significantly lowered MDA content of blood serum as compared with group 1. Blood serum catalase fell after their joint administration, whereas it was 2.6 times as high in cutaneous homogenates after metformin. However, it decreased after melatonin [185].

#### 9.4. Uterine carcinogenesis

Forty-eight oophorectomized Balb/c mice were randomly assigned to receive saline, tamoxifen citrate (4 mg/kg), 17-beta estradiol hemihydrate (4 mg/kg), metformin (50 mg/kg), tamoxifen citrate (4 mg/kg) with metformin (50 mg/kg) or estradiol (4 mg/kg) with metformin (50 mg/kg) for 3 days. Histological markers of uterotrophy, including luminal epithelial cell height and density of endometrial glands, were quantified. Immunohistochemical expression of PCNA and S6K1 was evaluated. H-score was used for S6K1 expression [186]. Mice treated either with tamoxifen or estradiol had significantly increased density of endometrial glands and epithelial height compared to vehicle-only or metformin-only group ( $p < 0.001$ ). Addition of metformin to tamoxifen or estradiol treatment significantly decreased the density of endometrial glands and epithelial cell height ( $p < 0.05$ ). Addition of metformin to tamoxifen significantly decreased the H-score of S6K1 ( $p < 0.05$ ) and the immunohistochemical expression of PCNA ( $p < 0.05$ ) in uterine lining epithelium, glandular and stromal cells. Addition of metformin to estradiol significantly decreased the H-score of S6K1 ( $p < 0.05$ ) and the immunohistochemical expression of PCNA ( $p < 0.05$ ) in uterine-lining epithelium, glandular and stromal cells. The authors suggest that metformin appears to have antiproliferative effects on the endometrium of estradiol or tamoxifen-treated mice via inhibiting the mTOR-mediated S6K1 activation.

Treatment with metformin reduced the incidence (by 23%) and size (by 3 times) and increased (by 35%) the latency of cervicovaginal squamous-cell carcinomas induced by BP intravaginal applications in SHR mice [185]. Metformin significantly reduced mitotic activity, number of CD31<sup>+</sup> positive vessels and VEGF staining in these tumors. Benign vascular tumors of the uterus and ovarian haemangiomas developed most frequently in metformin-exposed female 129/Sv mice. Thus, *in toto* uterine and ovarian haemangiomas and haemangioendoteliomas were revealed respectively in 54.2% of control and 68.8% of metformin-exposed female mice,  $p < 0.03$  (Fisher's exact test). At the same time, the differences in the incidence of angiogenic tumors only of ovaries or utery between treated and untreated with metformin was not statistically significant [138].

#### 9.5. Hepatocarcinogenesis

A number of factors have been identified that increase the risk of hepatocellular carcinoma (HCC). Recently it has become appreciated that type-2 diabetes increases the risk of developing HCC. This represents a patient population that can be identified and targeted for cancer prevention. The biguanide metformin is a first-line therapy for the treatment of

type-2 diabetes in which it exerts its effects primarily on the liver. Studies linking metformin intake for the control of diabetes with a reduced risk of HCC suggests a role of metformin in HCC. Although a number of preclinical studies show the anticancer properties of metformin in various tissues, no studies have directly examined the effect of metformin on preventing carcinogenesis in the liver, one of its main sites of action.

Bhalla et al. [190] show in these studies that metformin protected mice against chemically induced liver tumors. Interestingly, metformin did not increase activation of AMPK, a known target of metformin. Rather, metformin decreased the expression of several lipogenic enzymes and lipogenesis. In addition, restoring lipogenic gene expression by ectopic expression of a transcription factor SREBP1c rescues metformin-mediated growth inhibition. This mechanism of action suggests that metformin may also be useful for patients suffering from other disorders associated with HCC in which lipid synthesis is increased. As a whole, these studies show that metformin prevents HCC and that it should be evaluated as a preventive agent for HCC in readily identifiable, at-risk patients.

### 9.6. Pancreatic carcinogenesis

Schneider et al. [191] examined whether the prevention of islet cell proliferation can inhibit the promotional effect of a high-fat diet in pancreatic carcinogenesis. Two groups of high-fat-fed hamsters were used. One group received metformin in drinking water for life (HF +Met group), and the other group served as a control (HF group). At the time when the normalization of the plasma insulin level was expected, all hamsters were treated with the pancreatic carcinogen, N-nitrosobis-(2-oxopropyl)amine. The experiment was terminated 42 weeks later. Although 50% of the hamsters in the high-fat group developed malignant lesions, none was found in the HF+Met group ( $P < 0.05$ ). Also, significantly more hyperplastic and premalignant lesions, most of which were found within the islets, were detected in the high-fat group (8.6 lesions/hamster) than in the HF+Met group (1.8 lesions/hamster). The authors stressed that the results lend further support to islet cells' significant role in pancreatic carcinogenesis and may explain the association between pancreatic cancer and obesity, which is usually associated with peripheral insulin resistance.

CD1 mice were randomized into five groups, receiving a high-carbohydrate, high-fat (HC-HF) hyperinsulinemia-inducing diet with 50, 100 or 250mg/kg body weight (mg/kg) metformin in drinking water, a standard diet or HC-HF diet alone. Animals on the HC-HF diet developed obesity and insulin resistance. They had significantly higher body weight, upper-normal fasting blood glucose, higher insulin secretion and utilization, and fatty degeneration of the liver. Metformin at the doses employed significantly reduced food and water consumption; however, only a dose of 250 mg/kg significantly reduced body weight gain and suppressed gluconogenesis and produced a remarkable reduction in insulin secretion. There was no observed metformin-related hepato-toxicity in any of the groups. In summary, metformin at various doses exhibits protective effects on the metabolic disorder caused by the HC-HF diet, with the most effective protection at a dose of 250mg/kg. These effects may explain its translational role relating to its anti-neoplastic potential in the human [192].

## 9.7. Digestive tract carcinogenesis

Vitale-Gross et al. [193] observed that metformin prevented the development of head and neck squamous cell carcinoma (HNSCC) induced with 4-NQO by significantly reducing the size and number of carcinogen-induced oral tumoral lesions and by preventing their spontaneous conversion to squamous cell carcinomas.

Treating rats with 1,2-dimethylhydrazine (DMH) (once a week for 4 weeks) caused the decrease in the level of biogenic amines, particularly dopamine in the hypothalamus, alongside a decrease of glucose tolerance and an increase in insulin and triglyceride blood levels. The exposure to DMH also caused inhibition of lymphocyte blastogenic response to phytohemagglutinin and lipopolysaccharide; a decrease in the level of antibody produced against sheep erythrocytes; and a decrease in phagocytic activity of macrophages [198]. Administration of phenformin started from the first injection of the carcinogen restored all of the above-mentioned immunological indices and inhibited DMH-induced colon carcinogenesis [198,199]. It is worthy to note that colon 38 adenocarcinoma growth was significantly inhibited in liver-specific IGF-1-deficient mice, whereas injections with recombinant human IGF-1 promoted tumor growth and metastases [203].

Levels of advanced glycation end products (AGE) and receptors for AGE (RAGE) were examined in azoxymethane (AOM)-injected Fischer 344 rats fed a control diet (Group C), a 15% linoleic acid (LA) diet (Group L), a control diet with 10% glucose drink (Group G), and a 15% LA diet with 10% glucose drink (Group L + G). Group L+G showed the most pronounced increase of body weight, blood sugar and serum insulin [197]. The rats in Group L+G showed the most pronounced multiplicity of aberrant crypt foci (ACF) and carcinomas with increased mucosal RAGE and AGE. IEC6 rat intestinal epithelial cells treated with AGE showed increased RAGE expression, which was inhibited by treatment with metformin. In the AOM-injected rat colon cancer model, treatment with metformin suppressed the levels of RAGE and AGE, as well as the multiplicity of ACF and carcinomas in Group L+G rats.

The LKB1 tumor suppressor phosphorylates and activates AMPK (AMP-activated protein kinase) when cellular energy levels are low, thereby suppressing growth through multiple pathways, including inhibiting the mTORC1 (mammalian target of rapamycin complex 1) kinase that is activated in the majority of human cancers. Blood glucose-lowering type-2 diabetes drugs also induce LKB1 to activate AMPK, indicating that these compounds could be used to suppress growth of tumor cells. Huang et al. [194] investigated the importance of the LKB1-AMPK pathway in regulating tumorigenesis in mice resulting from deficiency of PTEN (phosphatase and tensin homologue deleted on chromosome 10) tumor suppressor, which drives cell growth through overactivation of the Akt and mTOR (mammalian target of rapamycin) kinases. It was shown that inhibition of AMPK resulting from a hypomorphic mutation that decreases LKB1 expression does not lead to tumorigenesis on its own, but markedly accelerates tumor development in PTEN(+/-) mice. In contrast, activating the AMPK pathway by administering metformin, phenformin or A-769662 to PTEN (+/-) mice significantly delayed tumor onset. LKB1 is required for activators of AMPK to inhibit mTORC1 signalling as well as cell growth in PTEN-deficient cells. Pharmacological inhibition of LKB1 and/or AMPK would be undesirable, at least for the treatment of cancers

in which the mTORC1 pathway is activated. Most importantly, these results demonstrate the potential of AMPK activators — such as clinically approved metformin — as anticancer agents that will suppress tumor development by triggering a physiological signalling pathway that potently inhibits cell growth.

Tomimoto et al. [195] investigated the effect of metformin on the suppression of intestinal polyp formation in Pac (Min/+) mice. Administration of metformin (250 mg/kg) did not reduce the total number of intestinal polyps, but it significantly reduced the number of polyps larger than 2 mm diameter in Pac (Min/+) mice. To examine the indirect effect of metformin, the index of insulin resistance and serum lipid levels in Pac (Min/+) mice were assessed. These factors were not significantly attenuated by the treatment with metformin, indicating that the suppression of polyp growth is not due to the indirect drug action. The levels of tumor cell proliferation as determined by 5-bromodeoxyuridine and proliferating cell nuclear antigen immunohistochemical staining and apoptosis, via transferase deoxytidyl uridine end labeling staining in the polyps of metformin-treated mice, were not significantly different compared to those of control mice. Gene expression of cyclin D1 and c-myc in intestinal polyps were also not significantly different between those two groups. In contrast, metformin activated AMPK in the intestinal polyps, resulting in the inhibition of the activation of mammalian target of rapamycin, which plays an important role in the protein synthesis machinery [195].

Seven-week-old BALB/c mice were intraperitoneally (i.p.) injected with azoxymethane (AOM, 10 mg/kg) and then treated with or without metformin (250 mg/kg/d) for six weeks (for the investigation of aberrant crypt foci [ACF] formation) or 32 weeks (for polyp formation) [196]. The authors next investigated colonic epithelial proliferation using bromodeoxyuridine (BrdU) and the proliferating cell nuclear antigen (PCNA) labeling indices. Furthermore, to examine the indirect effect of metformin, the insulin resistance status and the serum lipid levels were assessed. Treatment with metformin significantly reduced ACF formation. The inhibitory effect of metformin on colon polyps was relatively modest. No significant differences in body weight or glucose concentration were observed. The BrdU and PCNA indices decreased in mice treated with metformin. Western blot analysis revealed that the phosphorylated mTOR, S6 kinase and S6 protein levels in the colonic mucosa decreased significantly in mice treated with metformin. The authors believe that metformin suppresses colonic epithelial proliferation via the inhibition of the mTOR pathway through the activation of AMPK.

### 9.8. Lung carcinogenesis

A/J mice were treated with oral metformin after exposure to the tobacco carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) [188]. Metformin reduced lung tumor burden by up to 53% at steady-state plasma concentrations that are achievable in humans. mTOR was only modestly inhibited in lung tumors. To test whether intraperitoneal administration of metformin might improve mTOR inhibition, we injected mice and assessed biomarkers in liver and lung tissues. Plasma levels of metformin were significantly higher than oral administration after injection. In liver tissue, metformin activated AMPK and inhibited mTOR. In lung tissue, metformin did not activate AMPK but inhibited

phosphorylation of IGF-I receptor/insulin receptor (IGF-1R/IR), Akt, extracellular signal-regulated kinase (ERK) and mTOR. This suggested that metformin indirectly inhibited mTOR in lung tissue by decreasing activation of IGFIR/IR and Akt upstream of mTOR. Based on these data, the authors repeated the NNK-induced lung tumorigenesis study using intraperitoneal administration of metformin. Metformin decreased tumor burden by 72%, which correlated with decreased cellular proliferation and marked inhibition of mTOR in tumors. These studies show that metformin prevents tobacco carcinogen-induced lung tumorigenesis [188].

Sixty-one male 129/Sv mice were exposed to a single intraperitoneal injection of urethane dissolved in 0.9% neutral saline at the dose of 1g/kg of the body weight. Starting the day after the carcinogen injection, mice of one group were given 5 days a week of metformin with drinking water at a dose of 200 mg/l. Control mice were given drinking water without metformin. Six months after the urethane treatment, the experiment was stopped and all mice were sacrificed. Tumors (lung adenomas and thymic lymphomas) developed in 29 of 30 control mice (96.7%) and in 25 of 31 mice exposed to metformin (80.7%;  $p < 0.05$ ). Solid or trabecular lung adenomas developed in 90% of the control mice and in 77% of the mice in the group given metformin ( $p < 0.119$ ) [189]. These data show the inhibitory effect of metformin on urethane-induced tumorigenesis in mice.

### 9.9. Neurogenic and kidney transplacental carcinogenesis

A decrease of glucose utilization in the oral glucose tolerance test was found in the 3-month-old female progeny of rats exposed to NMU on the 21<sup>st</sup> day of pregnancy [201]. The serum insulin level was not different from the control, but the cholesterol level was higher in offspring of NMU-treated rats as compared with the control. Postnatal treatment with buformin started from age 2 months significantly inhibited the development of malignant neurogenic tumors in rats transplacentally exposed to NMU. Similar results have been observed in rats exposed transplacentally to N-nitrosoethylurea (NEU) and postnatally to phenformin [200]. The authors observed decreased development of nervous system and renal tumors induced transplacentally with NEU.

### 9.10. Radiation carcinogenesis

The treatment with phenformin also inhibited the carcinogenesis induced by a single total-body X-ray irradiation in rats [202].

Thus, antidiabetic drugs inhibit spontaneous and induced carcinogenesis in rodents. The effect did not depend on type of a carcinogen, tumor localization and histogenesis: it was observed in the relation of epithelial, mesenchymal, neurogenic and lymphoid malignancies developed spontaneously or induced by various chemical carcinogens (polycyclic aromatic hydrocarbons, nitroso compounds), irradiation, virus, transgene.

## 10. Antidiabetic drugs and cancer risk in diabetic patients

There is evidence that insulin resistance or some other aspect of type-2 diabetes may promote breast and some other cancers [109,203-205]. Biguanides were used as a component of the so-called metabolic rehabilitation of breast and colon cancer patients



[109,122,123]. The total of 324 patients (182 with breast cancer and 142 with colon cancer) treated by surgery of the primary tumor were randomly divided into control and treatment groups. In the latter group, a diet low in saturated fats and cholesterol was combined with treatment with biguanides or hypolipidemic drugs (mainly clofibrate in breast cancer patients). Among patients treated with biguanides, 304 were given the drug for more than 3 years and 15% for more than 5 years. The authors reported an overall improvement in the cumulative survival by 3-6 and 4-7 years of observation in groups with breast and colon cancer, respectively, as well as a slight decrease in frequency of primary multiple neoplasms and metachronous tumors in the contralateral breast [109,122-127]. Evans et al. [206] reported results of a pilot case-control study on 11,876 diabetic patients treated or not treated with metformin. The authors collated the information about use of metformin for all cases and controls and calculated the unadjusted odds ratio of cancer risk using conditional logistic regression. More than a third (336; 36.4%) of the subjects had been given at least one prescription of metformin in the year before their index date compared with 732 (39.7%) of the controls. The unadjusted odds ratio of cancer risk was estimated as 0.86 (95% confidence interval, 0.73 to 1.02). The unadjusted odds ratio for any exposure to the drug since 1993 was 0.79 (0.67 to 0.93). It is worthy to note that the authors suggested a dose-response relationship: adjusted cancer risk odds ratio for patients dispensed total amount 14-672 g of metformin was 0.83 (0.65 to 1.06); dispensed 673-964 g of metformin — 0.86 (0.68 to 1.10); and dispensed more than 964 g — 0.57 (0.43 to 0.75).

In another study, cancer-related mortality was compared among inception cohorts of metformin users and sulfonylurea monotherapy users [207]. There were 10,309 new users of metformin or sulfonylurea with an average follow-up of  $5.4 \pm 1.9$  years. Cancer mortality over follow-up was 4.9% (162 of 3,340) for sulfonylurea monotherapy users, 3.5% (245 of 6,969) for metformin users ( $p = 0.01$  for  $\chi^2$  test) and 5.8% (84 of 1,443) for patients who used insulin. Multivariate adjustment has shown that the sulfonylurea cohort had greater cancer-related mortality compared with the metformin cohort (1.3 (95% CI 1.1-1.6);  $p = 0.012$ ), whereas insulin use was associated with an adjusted cancer-related mortality of 1.9 (1.5 – 2.4;  $p < 0.0001$ ).

Cochrane report stressed that there were either insufficient or no data on the relative efficacy of metformin for preventing the development of diabetes, cardiovascular disease or endometrial cancer [208].

## 11. Mechanisms of effects of antidiabetic biguanides on aging and cancer

It is noteworthy that studies of metformin distribution in mice showed a measurable accumulation of  $^{14}\text{C}$  metformin in brain tissue two hours after a single oral dose (150 mg/kg) [209]. The studies revealed that orally administered metformin increases brain AMPK activation [142]. This suggests that metformin crosses the blood-brain barrier and exerts a pharmacological effect in the intact brain.

Data on molecular mechanisms of the inhibitory effect of biguanides on tumor growth have been discussed in several recent papers [174,175,210]. The formation and accumulation of advanced glycation end products (AGE) in various tissues are known to be involved in the

aging process and complications of long-term diabetes [106,107]. Effects of biguanides buformin and metformin on AGE formation have been studied *in vitro* [211]. It was shown that both drugs are potent inhibitors of AGE formation. In a descriptive clinical trial, 6-month-long metformin treatment of women with polycystic ovary syndrome (PCOS) significantly reduced AGE levels [212]. The AGE formation contributes to atherogenic oxidative modification of low-density lipoproteins (LDL). The impact of glycemic control on the parameters of free radical oxidation has been studied in type-2 diabetic patients treated with metformin or sulfonylurea [213]. Metformin was more effective than sulfonylurea drugs in decreasing glycosylated hemoglobin (Hb A1c), lipid peroxidation and malonic dialdehyde levels in the serum, and increasing SOD and glutathione peroxidase in erythrocytes. There is evidence that metformin primarily inhibits complex I of the mitochondrial respiratory chain [214-216]. In mice, metformin treatment protected against adriamycin-induced increase in the serum level of malonic dialdehyde and a decrease in the serum level of glutathione [217].

A number of reports concerning the cellular response to and molecular function of metformin have been published. Metformin has been shown to activate AMP-activated protein kinase [131] and inhibit the tyrosine phosphatase activity [218]. Metformin was reported to influence hepatic gene expression in cultured hepatocytes, including alterations in the expression of glucose-6-phosphatase (*G6pc*), gluco-kinase and 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2 (*Hmgcs2*). These genes, which are related to glycolysis-glyconeogenesis or cholesterol metabolism, were assumed to be critical for the action of metformin [219]. Global gene expression analysis was performed in livers of obese diabetic *db/db* mice 3 hours after a single administration of metformin (400 mg/kg) [220]. It identified 14 genes that showed at least a 1.5-fold difference in expression following metformin treatment, including a reduction of glucose-6-phosphatase expression. It was recently observed that the antidiabetic biguanides metformin and phenformin inhibit mTORC1 signaling, not only in the absence of TSC1/2 but in the absence of AMPK, instead dependent on the Rag GTPases [221].

Spindler [222] found that 8 weeks of metformin treatment was superior to 8 weeks of caloric restriction in terms of reproducing the specific changes in transcript levels produced by long-term caloric restriction in the liver of mice. The gene expression changes common to metformin and CR were associated with xenobiotic metabolism, cellular stress, energy metabolism, biosynthesis, signal transduction and the cytoskeleton [223]. It was suggested that antidiabetic biguanides inhibit metabolic immunodepression, which develops in animals exposed to carcinogenic agents and resembles immunodepression inherent to normal aging and specific age-related pathology [109,110]. If immunodepression is one of the important factors in carcinogenesis, then the elimination of metabolic immunodepression, which arises in the course of normal aging or under the influence of chemical carcinogens or ionizing radiation, can provide an anticarcinogenic prophylactic effect [109,110,122,198].

Although it is known that free radicals are produced during metabolic reactions, it is largely unknown which factor(s), of physiological or pathophysiological significance, modulate their production *in vivo*. It has been suggested that hyperinsulinemia may increase free radicals and therefore promote aging independent of glycemia [107,109]. Plasma levels of

lipid hydroperoxides are higher, and antioxidant factors are lower, in individuals who are resistant to insulin-stimulated glucose disposal but otherwise glucose tolerant, nonobese and normotensive [107]. This finding indicates that enhanced oxidative stress is present before diabetes ensues and therefore cannot simply be explained by overt hyperglycemia. There is substantial evidence supporting the hypothesis that selective resistance to insulin-stimulated (muscle) glucose disposal and the consequential compensatory hyperinsulinemia trigger a variety of metabolic effects, likely resulting in accelerated oxidative stress and aging [107,109].

The antidiabetics biguanides inhibit fatty acid oxidation and gluconeogenesis in the liver; increase the availability of insulin receptors; decrease monoamine oxidase activity [120,121]; increase sensitivity of hypothalamo-pituitary complex to negative feedback inhibition; and reduce excretion of glucocorticoid metabolites and dehydroepiandrosteronesulfate [109]. These drugs have been proposed for prevention of the age-related increase of cancer and atherosclerosis, and for retardation of the aging process [109,128]. It has been shown that administration of antidiabetic biguanides to patients with hyperlipidemia lowers the level of blood cholesterol, triglycerides and  $\beta$ -lipoproteins. Biguanides also inhibit the development of atherosclerosis and reduce hyperinsulinemia in men with coronary artery disease. Moreover, these drugs increase hypothalamo-pituitary sensitivity to inhibition by dexamethasone and estrogens; cause restoration of estrous cycle in persistent-estrous old rats; improve cellular immunity in atherosclerotic and cancer patients; and lower blood IGF-1 levels in cancer and atherosclerotic patients with type IIb hyperlipoproteinemia. Recently, it was shown that metformin decreases platelet superoxide anion production in diabetic patients [224]. There are observations that diablenol has similar effects [225]. Metformin increased glutathione (GSH) levels and diminished lipid peroxidation of T cells in mice; thus, the decrease of oxidative stress inhibited proliferation of T cells [226].

Morales et al. [227] showed that metformin ameliorates gentamicin-induced kidney injury through a mitochondria-dependent pathway, normalizing oxidative stress and restoring mitochondrial functional integrity [228]. It was shown that a single oral administration of metformin (100, 500 or 2500mg/kg) or multiple (4 or 8 weeks) treatments (100 or 500  $\mu$ g/kg) were not toxic or cytotoxic in streptozotocin-induced diabetic or control non-diabetic rats. Moreover, metformin protected rat bone marrow and sperm cells against diabetes-induced genomic instability and decline in cell proliferation [229]. Pretreatment with metformin reduced the doxorubicin-induced high frequency of micronuclei and protected against the doxorubicin-induced alteration of MDA and GSH in mice [217].

The available data on gender-specificity of the lifespan-extending effect of metformin will require further studies. It is worthy to note that metformin is used clinically to treat hyperandrogenemia in women with polycystic ovary syndrome. Insulin resistance in these patients contributes to the increased levels of free androgen. Metformin, as an insulin sensitizing agent, is an effective treatment [230].

It was shown in mice that deletion of ribosomal S6 protein kinase 1 (*S6K1*), a component of the nutrient-responsive mTOR (mammalian target of rapamycin) signaling pathway, led to increased lifespan and resistance to age-related pathologies such as bone, immune and motor

dysfunction, as well as loss of insulin sensitivity [56]. Deletion of *S6K1* induced gene expression patterns similar to those seen in CR or with pharmacological activation of adenosine monophosphate (AMP)-activated protein kinase (AMPK), a conserved regulator of the metabolic response to CR [56]. It is noteworthy that the mean lifespan extension was observed in female (+20.4%) but not in male *S6K1*<sup>-/-</sup> mice. The mean lifespan of oldest 10% survivors and maximum lifespan were also increased only in females. There was no difference in the incidence of macroscopic tumors in *S6K1*<sup>-/-</sup> and wild-type mice.

The strain differences in susceptibility to metformin could also be a reason of variability in response to treatment. It was shown that mean strain-specific lifespan varied 2- to 3-fold under *ad libitum* feeding and 6- to 10-fold under dietary restriction (DR) in virgin males and females in 41 recombinant inbred strains of mice [231]. Notably, DR shortened lifespan in more strains than those in which it lengthened life. Food intake and female fertility varied markedly among strains under *ad libitum* feeding, but neither predicted dietary-restricted mice survival. Therefore, strains in which DR shortened lifespans did not have low food intake or poor reproductive potential. These results demonstrate that life extension by DR may not be universal.

## 12. Conclusions

Research conducted during the last 15-20 years firmly established insulin, insulin-like and homologous signaling as key regulators of aging and longevity in organisms ranging from yeast to mammals. Disregulation of insulin signaling and carbohydrate homeostasis in diabetes produces numerous aging-like symptoms including increased risk of cancer and other age-related disease. Against this background, the potential role of some of the anti-diabetic drugs and genetic lifestyle modifications of insulin signaling in cancer prevention is of considerable scientific, clinical and public-health interest.

In a comment by G. Taubes in “Cancer prevention with a diabetic pill?,” published on January 6, 2012, in *Science* [232], it was stressed that “diabetics treated with metformin have from 25% to 40% less cancer than those who receive insulin as therapy or take sulfonylurea drugs that increase insulin secretion from the pancreas. Metformin already may have saved more people from cancer death than any drug in history. Some 120 million prescriptions are written for it yearly.” There is an exponential growth of publications on metformin and cancer. The comparison of the outcomes of these two approaches to cancer and aging prevention (genetic modifications and pharmacological interventions) should be helpful for extrapolation of experimental findings to clinical practice.

Striking similarities have been described between insulin/IGF-1 signaling pathways in yeast, worms, flies and mice [34]. Many characteristics of mice that are long lived due to genetic modifications resemble effects of caloric restriction in wild-type (normal) animals. Comparison of characteristics of animals exposed to these endogenous and exogenous influences shows a number of similarities and differences. Antidiabetic biguanides seem to be more effective than caloric restriction and some of the genetic manipulations in preventing age-related deterioration of insulin levels. This raises the intriguing possibility that these or related drugs may act as a targeted therapy in breast cancer. Metformin use in

diabetic patients has been associated with reduced cancer glucose metabolism and insulin signaling pathway, as well as such important longevity-related parameters as fertility and resistance to oxidative stress and tumorigenesis. Furthermore, there is evidence that metformin may have insulin-independent direct effects on cancer cells, acting as a mammalian target of rapamycin (mTOR) inhibitor [175,233-235]. It was suggested that this dual action of metformin (insulin reduction and mTOR inhibition) makes it a particularly attractive target for evaluation in breast cancer [175,236] as well as some other cancers, e.g. colon cancer. It remains to be shown whether antidiabetic biguanides extend lifespan independently of CR [25].

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## Biographies

Prof. **Vladimir N. Anisimov** was graduated as M.D. from the 1st I.P. Pavlov Leningrad Medical Institute (1968), received his PhD. (1972) and D.Sc. (1984) from the N.N. Petrov Research Institute of Oncology (St.Petersburg), since 1996 - Professor in Oncology. In 2011, he was elected as a Corresponding member of the Russian Academy of Sciences. Since 1987, he is Chief, Laboratory of Carcinogenesis and Aging at the Petrov's Institute, and since 1998 – also the Head, Department of Carcinogenesis and Oncogerontology at the same institute. His main scientific interests are relationship between aging and cancer, modifying factors of carcinogenesis, experimental gerontology. He is a member of numerous of scientific societies and of editorial board of several international journals, since 1964 he is the president, The Gerontological Society of the Russian Academy of Sciences. Prof. Anisimov is author and co-author of 20 monographs, including “Carcinogenesis and Aging”, Vols. 1 & 2, CRC Press, Boca Raton, 1987; “Principles for Evaluating Chemical Effects on the Aged Population (Environmental Health Criteria 144”. Geneva: W.H.O., 1993; “Cancer in the Elderly”, St.Petersburg: N.-L., 2004; “Molecular and Physiological Mechanisms of Aging” 2<sup>nd</sup> ed., Vol. 1& 2, St.Petersburg: Nauka, 2008, several chapters in books, more than 400 scientific papers in peer-reviewed international and Russian journals.

**Andrzej Bartke** is professor of Internal Medicine and Physiology at Southern Illinois University (SIU) School of Medicine in Springfield, Illinois, USA. The focus of his research is on the genetic and hormonal control of aging in mammals. Current work is aimed at identifying mechanisms that link reduced growth hormone action with delayed aging and extended longevity. For this work, he is using mutant mice that live longer than normal mice and show various symptoms of delayed aging, including retention of cognitive function and protection from age-related disease. His career includes work at the Jagiellonian University in Krakow, Poland, Worcester Foundation for Experimental Biology in Shrewsbury, Massachusetts, the University of Texas Health Science Center at San Antonio, and at SIU-Carbondale. He is a past president of the Society for the Study of Reproduction, the American Society of Andrology and the American Aging Association. Dr. Bartke has published more than 500 research papers, review articles and book chapters dealing with reproductive endocrinology, prolactin, growth hormone and aging, and has received numerous awards, in addition to 40 years of continuous NIH funding.

**Table 1**

Key phenotypic characteristics of three types of mice used to study impact of GH on aging. Arrows pointing up and down denote significant increases and decreases, respectively.

	<b>GHR-KO</b>	<b>Ames dwarf</b>	<b>Snell dwarf</b>
<b>Genetic alteration</b>	<b>GHR deletion</b>	<b>Prop1 mutation</b>	<b>Pit1 mutation</b>
Plasma GH		Not detectable	Not detectable
Plasma IGF-1			
Adult body size			
Plasma insulin			



	GHR-KO	Ames dwarf	Snell dwarf
Genetic alteration	GHR deletion	Prop1 mutation	Pit1 mutation
Blood glucose	↓ Or —	↓	↓
Plasma adiponectin	↑	↑	↑
Age of puberty	↑	↑	↑
Longevity	↑	↑	↑

**Table 2**

Effect of antidiabetic drugs on lifespan in mice and rats.

Strain	Sex	Treatment	No. of animals	Life span, days		References
				Mean	Last 10% of survivors Maximum	
Mice						
C3H/Sn	Female	Control	30	450 ± 23.4	631 ± 11.4	643
		Phenformin	24	545 ± 39.2 (+21.1%)	810 ± 0* (+28.4%)	810 (+26%)
FVB/N	Female	Control	34	264 ± 3.5	297 ± 7.3	311
		Metformin	32	285 ± 5.2 (+8.0%)	336 ± 2.7 (+13.1%)*	340 (+16.2%)
FVB/N	Female	Control	15	285 ± 12	396 ± 0	396
		Metformin	20	304 ± 10	352 ± 7	359
SHR	Female	Control	50	388 ± 29.2	727 ± 22.5	814
		Metformin	50	535 ± 31.9* (+37.9%)	878 ± 6.6* (+20.8%)	898 (+10.3%)
SHR	Female	Control	119	511 ± 20.3	881 ± 12.8	941
		Metformin	51	583 ± 26.7 (+14.1%)	897 ± 27.4*	972
129/Sv	Male	Control	41	662 ± 27.7	951 ± 32.3	1029
		Metformin	46	573 ± 26.5 (-13.4%)*	931 ± 30.4	1044
129/Sv	Female	Control	47	706 ± 20.8	910 ± 8.9	930
		Metformin	48	742 ± 16.3 (+5.1%)	913 ± 19.2	966 (+3.9%)
Rats						
LJO	Female	Control	41	652 ± 27.3	885 ± 11.3	919
		Phenformin	44	652 ± 28.7	974 ± 16.2** (+10.1%)	1009 (+9.8%)
Fischer-344	Female	Control	74	687 ± 19.2	925 ± 22.5	1054
		Buformin	42	737 ± 26.4 (+7.3%)	1036 ± 38.9* (+12%)	1112 (+5.5%)
Fischer-344	Male	Control	31	796 ± 170	1039 ± 29.6	1065
		Metformin	40	815 ± 186	1061 ± 2.5	1062

The difference with control is significant:

\* - p < 0.05;

\*\*\*  
p < 0.01 (Student's test)

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Table 3

Effect of antidiabetic biguanides on spontaneous and induced carcinogenesis in various target tissues of rodents.

Tumor target	Strain, species	Carcinogenic agent	Drug	Effect	References
Mammary gland	C3H/Sn mice	MMTV	Phenformin	Inhibition	133
	FVB/N mice	HER-2/neu	Metformin	Inhibition	134,135
	SHR mice	spontaneous	Metformin	No effect	136,137
	LJO rats	DMBA	Phenformin	Inhibition	176,177
	LJO rats	DMBA	Bufformin	Inhibition	178
	LJO rats	NMU	Phenformin	Inhibition	179
	SD rats	NMU	Metformin	Inhibition	180
	SD rats	NMU	Metformin	Inhibition	181
	SHR mice	Benzo(a)pyrene	Metformin	Inhibition	182,183
	Outbred mice	MCA	Pheformin	Inhibition	184
Soft tissues	SHR mice	Benzo(a)pyrene	Metformin	Inhibition	185
	129/Sv mice	Spontaneous	Metformin	Inhibition	138
Uterus	Balb/c mice	Estradiol, tamixifen	Metformin	Inhibition	186
	SHR mice	Benzo(a)pyrene	Metformin	Inhibition	187
Endometrium	A/J mice	NNK	Metformin	Inhibition	188
	129/Sv mice	Urethan	Metformin	Inhibition	189
Cervix uteri and vagina	C57BL/6 mice	DENA	Metformin	Inhibition	190
	Hamsters	NBOPA	Metformin	Inhibition	191
Liver	CD1 mice	High carbohydrate fat diet	Metformin	Inhibition	192
	C57BL/6 mice	4-NQO	Metformin	Inhibition	193
Pancreatic islets	PTEN+/- mice	Spontaneous	Metformin	Inhibition	194
	PTEN+/- mice	Spontaneous	Phenformin	Inhibition	194
Oral mucosa	PTEN+/- mice	Spontaneous	Metformin	Inhibition	195
	<i>Apc<sup>Min/+</sup></i> mice	Spontaneous	Metformin	Inhibition	196
Intestines	Balb/c mice	Azoxymethane	Metformin	Inhibition	197
	F344 rats	Azoxymethane	Metformin	Inhibition	198,199
Small intestines	LJO rats	DMH	Phenformin	Inhibition	198,199
	LJO rats	NEU, tp	Bufformin	Inhibition	200

Tumor target	Strain, species	Carcinogenic agent	Drug	Effect	References
Lymphoid tissue	PTEN <sup>+/-</sup> mice	Spontaneous	Metformin	Inhibition	194
	PTEN <sup>+/-</sup> mice	Spontaneous	Phenformin	Inhibition	194
Nervous system	LJO rats	NMU, tp	Buformin	Inhibition	201
	LJO rats	NEU, tp	Buformin	Inhibition	200
Total tumors	129/Sv mice	Spontaneous	Metformin	Inhibition	138
	LJO rats	Spontaneous	Buformin	Inhibition	140
	LJO rats	Spontaneous	Phenformin	Inhibition	139,202
	LJO rats	X-rays	Phenformin	Inhibition	139
	LJO rats	X-rays	Phenformin	Inhibition	139

B(a)P - Benzo(a)pyrene; DMBA - 7,12-dimethylbenz(a)anthracene; DMH - 1,2-dimethylhydrazine; E2 - estradiol-17β; MCA - 20-methylcholanthrene; MMTV - murine mammary tumor virus; NBOPA - N-nitrosobis(2-oxopropyl) amine; NEU - N-nitrosoethyl-urea; NMU - N-nitrosomethylurea; NNK - 4-(methylnitroamino)-1-(3-pyridyl)-1-butanone; 4-NQO - 4-nitroquinoline-1-oxide.