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Research article

Insulin and longevity: antidiabetic biguanides as geroprotectors

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Abstract

The results of previous experimental studies of effects of antidiabetic biguanides (phenformin and buformin) on life span and spontaneous tumor incidence in mice and rats were recalculated and reanalyzed using standard demographic models of mortality. The chronic treatment of female C3H/Sn mice with phenformin prolonged the mean life span by 21.1% ($P < 0.05$), the mean life span of the last 10% survivors by 28.4% and the maximum life span by 5.5 months (by 26%) in comparison with the control. The demographic aging rate represented by the estimate of respective Gompertz's parameter decreased by 31.2% and MRDT increased 1.45-fold. The treatment significantly inhibited (4.0-fold, $P < 0.01$) the incidence of mammary adenocarcinomas in mice. Administration of phenformin to female LIO rats failed to influence the mean life span. At the same time, the mean life span of the last 10% survivors increased by 10.1% ($P < 0.05$), and maximum life span increased by 3 months (+9.8%). Phenformin attenuated the development of spontaneous tumors in comparison to the control. The treatment of female rats with another antidiabetic biguanide, buformin, slightly increased their mean life span (by 7.3%; $P > 0.05$). The mean life span of the last 10% survivors increased by 12% ($P < 0.05$) and the maximum life span increased by 2 months (+5.5%) as compared with controls. The population aging rate decreased by 18.1% ($P < 0.05$) and MRDT increased 1.22-fold under the influence of buformin ($P < 0.05$). The total tumor incidence decreased by 49.5% in buformin-treated rats. Both antidiabetic biguanides slightly decreased the body weight, slowed down the age-related decline of the reproductive function in female rats. The results of our experiments provide evidence that antidiabetic biguanides are promising geroprotectors as well as drugs which can be used in the prevention of cancer.

Introduction

An important aim of the molecular gerontology today is to unveil the genetics basis of longevity in humans. To achieve this aim many experiments modulating longevity in mammals have been performed. Caloric restriction (CR) is the only known intervention in mammals that has been shown consistently to increase life span, reduce incidence and retard the onset of age-related diseases, including cancer and diabetes. CR has also been shown to increase the resistance to stress and toxicity, and maintain function and vitality in laboratory mammals of younger ages (Weindruch and Walford 1988; Yu 1994; Masoro 2000). Studies in CR rhesus monkeys have produced physiological responses strikingly similar to those observed in rodents (Roth et al. 1999; Ramsey et al. 2000; Mattson et al. 2003). Emerging data from these studies suggest that long-term CR will reduce morbidity and mortality in primates, and thus may exert beneficial 'anti-aging' effects in humans (Roth et al. 1999; Lee et al. 2001).

The crucial event of the action of CR is the reduction in the levels of insulin and insulin-like growth factor-1 (IGF-1) and also an increase insulin sensitivity in rodents (Weindruch and Walford 1988) as

well as in monkeys (Lane et al. 2000). In *C. elegans* and *D. melanogaster*, the mutation modification of genes operating in the signal transduction from insulin receptor to transcription factor *daf-16* (*age*-1, daf-2, CHICO, InR, etc.) are strongly associated with longevity (Kenyon 2001; Clancy et al. 2001; Tatar et al. 2001). Whole-genome analysis of gene expression during aging of nematode worm *C. elegans* provided a 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 (Lund et al. 2002).

Daf-2 and InR are structural homologues of tyrosine kinase receptors in vertebrata that includes the insulin receptor and the IGF-1 receptor. It was shown that in vertebrata the insulin receptor regulates energy metabolism whereas IGF-1 receptor promotes a growth. During the last few years a series of elegant experiments in mice and rats in which this or that key elements of the insulin/IGF-1 signaling pathway were genetically modified provide an evidence of the involvement of the system in the control of mammalian aging and longevity (Coschigano et al. 2000; Bartke and Turyn 2001; Flurkey et al. 2001; Dominici et al. 2000, 2002; Hsiesh et al. 2002a, b; Shimokawa et al. 2003; Bartke et al. 2003; Bluher et al. 2003; Holzenberger et al. 2003; Tatar et al. 2003). Recently 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. This is the first evidence showing that mutation located downstream to daf-16 in insulin signal transduction system is associated with longevity (Anisimov et al. 2001).

Hyperglycemia is an important aging factor involved in generation of advanced glycosylation end products (AGEs) (Ulrich and Cerami 2001; Facchini et al. 2000a; Elahi et al. 2002). Untreated diabetics with elevated glucose levels suffer many manifestations of accelerated aging, such as impaired wound healing, cataracts, vascular and microvascular damage (Dilman 1994). The accumulation of the AGE, pentosidine, is accelerated in diabetics and has been suggested to be a reliable biomarker of aging (Ulrich and Cerami 2001; Verzijl et al. 2001). 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 (Dilman 1978, 1994; Colangelo et al. 2002; Gupta et al. 2002).

The concept of CR mimetics is now being intensively explored (Hadley et al. 2001; Mattson et al. 2001; Weindruch et al. 2001). CR mimetics involves interventions that produce physiological and anti-aging effects similar to CR. Reviewing the available data on the benefits and adverse effects of calorie restriction and genetic modifications, Longo and Finch (2003) suggested three categories of drugs which may have potential to prevent or postpone age-related diseases and extend life span: drugs that (1) stimulate dwarf mutations and therefore decrease pituitary production of GH; (2) prevent IGF-1 release from the liver, or (3) decrease IGF-1 signaling by the action on either extracellular or intracellular targets. Several years ago, it was suggested to use biguanide anti-diabetics as a potential anti-aging treatment (Dilman 1971, 1978; Dilman and Anisimov 1980). The anti-diabetic drugs, phenformin, buformin, and metformin, were observed to reduce hyperglycemia and produce the following effects: improved glucose utilization; reduced free fatty acid utilization, gluconeogenesis, serum lipids, insulin and IGF-1, and reduced body weight both in humans and experimental animals (Dilman 1994). At present, phenformin is not used in clinical practice due to its side effects observed in patients with non-compensated diabetes. It is worthy of note that during more than 10-years long experience of administration of phenformin for patients without advanced diabetes Dilman (1994) observed no cases of lactic acidosis or any other side effects. We believe that the analysis of results of long-term administration of this drug as well as another antidiabetic biguanides (buformin and metformin) to non-diabetic animals is very important for better understanding of the links between insulin and longevity. In this paper we present the results of reevaluation and mathematical modeling of the earlier published results obtained by Anisimov (1980, 1982) and Dilman and Anisimov (1980).

Material and methods

Three experiments

In experiment 1, female C3H/Sn mice kept from the age of 3.5 months at standard *ad libitum* diet were given phenformin (1-phenylethylbiguanide, Dibotin®, Winthrop Products Co., UK). Mice received phenformin 5 times a week orally with tube at a single dose of 2 mg/mouse in 0.2 ml of drinking water until a natural death (Dilman and

Table 1. Effect of antidiabetic biguanides on mortality rate and tumor incidence in mice and rats.

Species, strain	Treatment	No. of animals	Life span, days			No. of tumor-bearing animals
			Mean	Last 10% survivors	Maximum	
C3H/Sn mice	Control	30	450 ± 23.4	631 ± 11.4	643	24 (80.0%)
	Phenformin	24	545 ± 39.2*	810 ± 0**	810	5 (20.8%)[a]
			(+21.1%)	(+28.4%)	(+26%)	(–3.8-fold)
LIO rats	Control	41	652 ± 27.3	885 ± 11.3	919	18 (43.9%)
	Phenformin	44	652 ± 28.7	974 ± 16.2**	1009	12 (27.3%)
				(+10.1%)	(+9.8%)	(–1.6-fold)
	Control	74	687 ± 19.2	925 ± 22.5	1054	21 (28.4%)
	Buformin	42	737 ± 26.4	1036 ± 38.9*	1112	8 (19.0%)
			(+7.3%)	(+12%)	(+5.5%)	(–1.49-fold)

The difference with control is significant: $^{*}P < 0.05$; $^{**}P < 0.01$ (Student's *t*-test); $^{a}P < 0.05$ (Fischer's exact test).

Anisimov 1980). In experiments 2 and 3, female outbred LIO rats (Anisimov et al. 1989) were given phenformin (5 mg/rat/day) starting from the age of 3.5 month (experiment 2) or buformin (N-butylbiguanide, Adebit, Chynoin®, Hungary) (5 mg/rat/day) (experiment 3) in 1.0 ml of drinking water until a natural death (Anisimov, 1980, 1982). In all experiments the control group obtained corresponding amount of drinking water given orally with tube. Every month animals in experiments 2 and 3 were weighed. Once in every 3 months, vaginal smears taken daily for 2 weeks from the rats were cytologically examined to estimate the phases of their estrous functions. The date of each death was recorded, then the mean life span, the age by which 90% of the animals died and maximal life span, were estimated. All animals that died, or were sacrificed when moribund, were autopsied. At autopsy their skin and internal organs were examined. The tumors were microscopically examined. One can find other details of the experimental procedures in the above mentioned publications.

Statistics

Experimental results were statistically processed by methods of variation statistics (Goubler 1978). The significance of discrepancies was defined according to Student's *t*-criterion, Fischer's exact method, and χ^2-analysis (Goubler 1978). For survival analysis, Cox's method (Cox and Oakes 1996) was used. All reported test values for survival analyses are two sided.

Mathematical models and estimations

The mathematical model used to describe survival is the Gompertz model with the survival function

$$S(x) = \exp\left\{-\frac{\beta}{\alpha}[\exp(\alpha x) - 1]\right\}$$

where parameters α and β are associated with aging and initial mortality rate, respectively. Parameter α is often characterized by the value of mortality rate doubling time (MRDT), calculated as $\ln(2)/\alpha$. Parameters for the model were estimated from data using the maximum likelihood method implemented in the Gauss statistical system (Gauss System 1994). Confidence intervals for the aging rate parameter estimates were calculated using log-likelihood functions (Cox and Oakes 1994).

Results

The data on effect of antidiabetic biguanides on life span and spontaneous tumor incidence in mice and rats are given in Tables 1–3.

The chronic treatment of female C3H/Sn mice with phenformin prolonged mean life span by 21.1% ($P < 0.05$), the mean life span of last 10% survivors by 28.4% and the maximum life span by 5.5 months (by 26%) in comparison with the control (Table 1). At the time of death of the last mice in the control group 41.7% of phenformin-treated mice were alive. The demographic aging rate represented by the estimate of respective Gompertz's parameter was decreased by

Table 2. Parameters of life span in female C3H/Sn mice treated and not treated with phenformin.

Group	Total no. of cases	Tumor-bearing mice	Tumor-free mice
Number of mice			
Control	30	24 (80.0%)	6
Phenformin	24	5 (20.8%)[a]	19[a]
Mean life span (days)			
Control	450 ± 23.4	472 ± 25.1	362 ± 49.0
Phenformin	545 ± 39.4*	499 ± 111.6	557 ± 41.6**
Mean life span of the last 10% of survivors (days)			
Control	631 ± 11.4	642 ± 1.0	470
Phenformin	810 ± 0**	731	810
Aging rate $\alpha \times 10^3$ *(days*$^{-1}$*)*			
Control	7.64 (7.49; 8.10)	9.02 (8.88; 9.57)	8.41 (6.87; 9.79)
Phenformin	5.26 (4.94; 5.51)[#]	2.06 (1.40; 5.30)[#]	5.64 (5.63; 6.32)[#]
MRDT (days)			
Control	90.7 (85.6; 92.5)	76.8 (72.4; 78.1)	82.4 (70.8; 100.9)
Phenformin	131.8 (125.8; 140.3)[#]	336.9 (130.8; 495.1)[##]	122.9 (109.7; 123.1)[#]

Mean life spans are given as mean ± standard error; 95% confidence limits are given in parentheses; MRDT = mortality rate doubling time.
The difference with controls is significant: [a]$P < 0.05$ (Fischer's exact test); *$P < 0.05$; **$P < 0.01$ (Student's *t*-test); [#]$P < 0.05$ (Cox's method).

31.2% and MRDT increased by 1.45-fold. One can see from Figure 1A that treatment with phenformin significantly shifted survival curve to the right (P = 0.00136, log-rank test) and slowed down the aging rate. The treatment significantly inhibited (by 4.0-fold, $P < 0.01$) the incidence of mammary adenocarcinomas in the mice (Table 1) and significantly slowed down population aging rate for tumor-bearing mice and increased MRDT in them (Table 2). The tumor yield curve rise was also significantly slowed down as a result of the treatment (P = 0.0623, long-rank test) (Figure 1B).

Administration of phenformin to LIO rats failed influence the mean life span. At the same time, the mean life span of the last 10% survivors was increased by 10.1% (P <0.05), and maximum life span was increased by 3 months (+9.8%) in comparison with the controls (Table 1). The treatment with phenformin slightly decreased the body weight of rats in comparison with the control ($P > 0.05$) (data are not shown). The disturbances in the estrus function observed in 36% of 15–16-month old rats of the control group and only in 7% of rats in phenformin-treated group ($P < 0.05$). The population aging rate was decreased by 19.8% and MRDT increased by 1.28-fold under influence of phenformin, however these changes were not statistically significant ($P > 0.05$) (Table 3). Only 9% of phenformin-treated rats were alive at the time of the death of the last survivor in the control group. The calculated survival curves for control and phenformin-treated rats were practically similar (Figure 2A). Also statistically insignificant ($P > 0.05$) was 1.6-fold decrease in total spontaneous tumor incidence observed in phenformin-treated rats and failed influence the fatal tumor incidence in them. However, under the treatment significant inhibition of the total tumor yield curve gain was observed (Figure 2B).

The treatment of rats with another antidiabetic biguanide, buformin, slightly increased their mean life span (by 7.3%; $P > 0.05$). The mean life span of the last 10% survivors increased by 12% ($P < 0.05$) and the maximum life span increased by 2 months (+5.5%) as compared with controls. The treatment with buformin also increased the mean life span in both tumor-bearing and tumor-free rats (Table 3). The body weight of rats treated with buformin was slightly (5.2 to 9.4%) but statistically significantly ($P < 0.05$) decreased in comparison with the control from the

Table 3. Parameters of life span in female rats treated and not treated with phenformin and buformin.

Group	Total No. of cases	Tumor-bearing rats	Tumor-free rats
Number of rats			
Control-1	41	18 (43.9%)	23
Phenformin	44	12 (27.3%)	32
Control-2	74	21 (28.4%)	53
Buformin	42	8 (19.0%)	34
Mean life span (days)			
Control-1	652 ± 27.3	744 ± 35.0	564 ± 32.1
Phenformin	652 ± 28.7	716 ± 48.4	595 ± 30.0
Control-2	687 ± 19.2	717 ± 25.2	652 ± 22.1
Buformin	737 ± 26.4	745 ± 60.1	713 ± 25.4
Mean life span of the last 10% of survivors (days)			
Control-1	885 ± 11.3	778 ± 141.5	830 ± 18.0
Phenformin	974 ± 16.2**	750 ± 0	860 ± 28.0
Control-2	925 ± 22.5	828 ± 45.5	868 ± 1.9
Buformin	1036 ± 38.9*	927 ± 0*	931 ± 2.0**
Aging rate $\alpha \times 10^3$ ($days^{-1}$)			
Control-1	5.62 (5.30; 5.99)	9.41 (8.98; 9.85)	6.81 (6.57; 7.04)
Phenformin	4.39 (4.22; 6.66)	8.86 (8.12; 9.60)	6.41 (6.19; 6.64)
Control-2	6.90 (6.58; 6.99)	9.93 (9.68; 10.2)	7.18 (7.02; 7.35)
Buformin	5.65 (5.64; 6.27)#	7.97 (6.31; 9.63)#	7.70 (7.51; 7.90)#
MRDT (days)			
Control-1	123.3 (115.7; 130.8)	77.2 (70.4; 77.2)	101.8 (98.5; 105.5)
Phenformin	157.9 (104.1; 164.3)	78.2 (72.2; 85.4)	108.1 (104.4; 112.0)
Control-2	100.5 (99.2; 105.3)	69.9 (68.0; 71.6)	96.5 (94.3; 98.7)
Buformin	122.7 (110.6; 122.9)#	87.0 (72.0; 109.9)#	90.0 (87.7; 92.3)

Mean life spans are given as mean ± standard error; 95% confidence limits are given in parentheses; MRDT = mortality rate doubling time.
The difference with controls is significant: a$P < 0.05$ (Fischer's exact test); *$P < 0.05$ (Student's *t*-test); #$P < 0.05$ (Cox's method).

age of 12 months to 23 months ($P < 0.05$) (data are not shown). At the age of 16–18 months 38% of control rats revealed the disturbances in the estrus cycle persistent estrus, repetitive pseudopregnancies or anestrus), whereas in females treated with buformin these disturbances were observed only in 9% of rats ($P < 0.05$). The population aging rate was decreased by 18.1% ($P < 0.05$) and MRDT increased by 1.22-fold under influence of buformin ($P < 0.05$). Buformin also decreased the demographic aging rate calculated in both tumor-bearing and tumor-free rats (Table 3). Survival curve in the group of buformin-treated rats was slightly shifted to the right in comparison with the control (Figure 3A). The decrease in the total tumor incidence observed in buformin-treated rats (by 49.5%) was statistically insignificant in the Fischer's exact test. However, one can see in Figure 3B that the tumor yield gain in buformin-treated rats was inhibited by the treatment ($P = 0.133$, log-rank test).

Discussion

Calculations presented in this study confirmed early reported conclusion on geroprotective potential of biguanides (Anisimov 1980, 1982; Dilman and Anisimov 1980) and provide a new evidence of this effect. The results of three experiments with animal

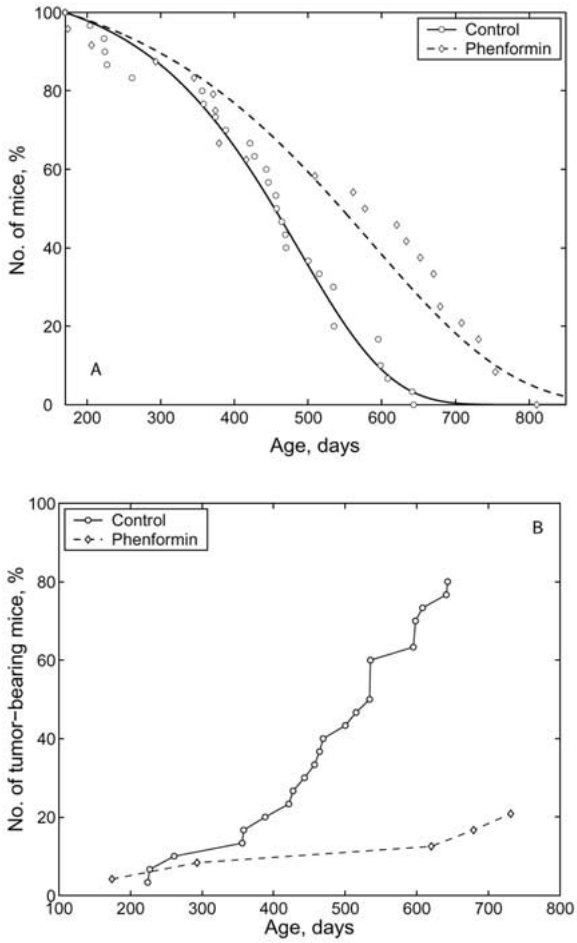

Figure 1. Effect of phenformin on the survival (A) and total tumor yield (B) curves in female C3H/Sn mice.

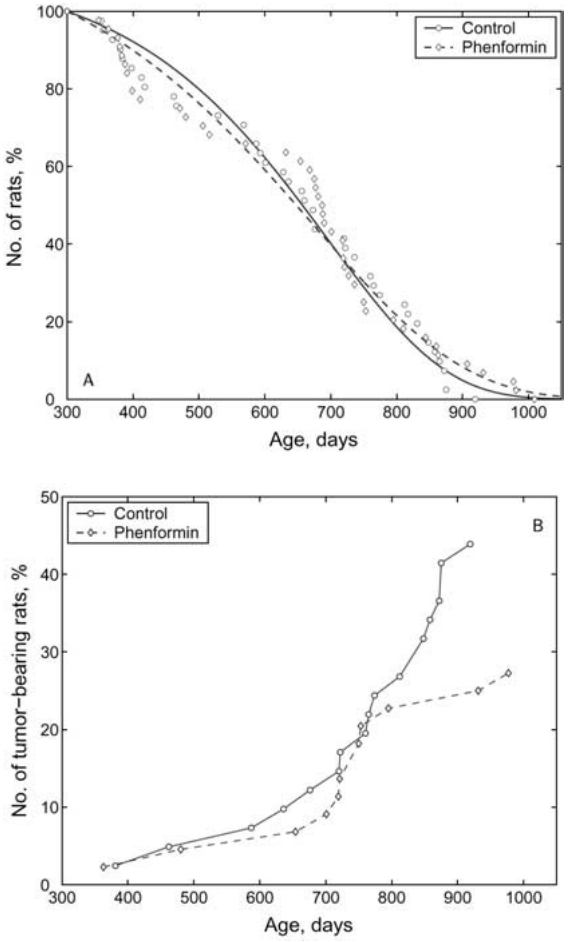

Figure 2. Effect of phenformin on the survival (A) and total tumor yield (B) curves in female LIO rats.

models show that antidiabetic biguanides slow down the demographic aging rate and prolong the mean life span. The increase was also observed in the mean life span among the last 10% of survivors, and in the maximum life span. Recently, the life span extension was observed in rats treated with metformin (G.S. Roth, personal communication). Bakaev et al. (2002) reported the increase in longevity of *C. elegans* exposed to buformin. It is worth noting that both phenformin and buformin slowed down the age-related switching-off the reproductive function. Several other effects of treatment with phenformin related to reproduction and aging, are known from earlier studies. For example, it decreased hypothalamic threshold of the sensitivity to feedback inhibition by estrogens, which is one of the most important mechanisms regulating age-related decline and switch-off of the reproductive function (Dilman and Anisimov 1979). It is worthy of note that metformin may improve menstrual regularity, leading to spontaneous ovulation, and enhance the induction of ovulation with clomiphene citrate in women with polycystic ovary syndrome (Awartani and Cheung 2002; Netsler et al. 2002). The treatment with phenformin also decreased hypothalamic threshold sensitivity to feedback regulation by glucocorticoids and by metabolic stimuli (glucose and insulin) (Dilman et al. 1979a, b; Dilman 1994). It was recently shown that elements involved in the insulin-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 (Fernandes et al. 2001) and brain's control of life span (Mattson 2002; Mattson et al. 2002). Antidiabetic biguanides also alleviated age-related

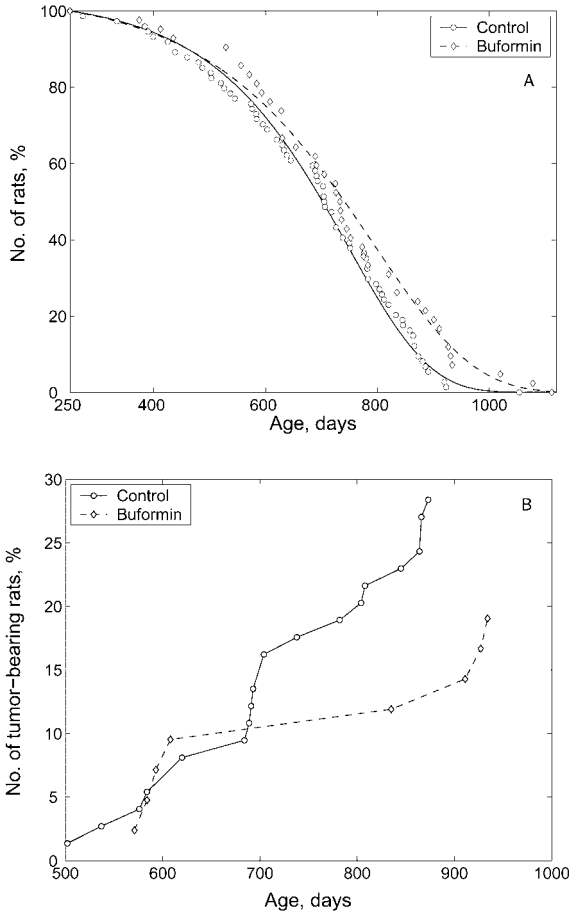

Figure 3. Effect of buformin on the survival (A) and total tumor yield (B) curves in female LIO rats.

metabolic immunodepression (Dilman 1994). These mechanisms can be involved in geroprotector effect of biguanides. Treatment with chromium picolinate which elevated the insulin sensitivity in several tissues, including hypothalamus, significantly increased the mean life span and decreased the development of age-related pathology in rats (McCarty 1994).

In all three experiments antidiabetic drugs inhibited the development of spontaneous tumors. This effect was more pronounced in mice compared to rats. However, this may be the effect of dose: calculations show that the daily dose of phenformin in mice was in the ranges 60–80 mg/kg of the body weight, whereas in rats –30–50 mg/kg of body weight. The anticarcinogenic effect of antidiabetic biguanides has been demonstrated in several models of carcinogenesis (Anisimov 1987; Schneider et al. 2001).

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 (Dilman 1971, 1994; Facchini et al. 2000a, 2001). Plasma levels of lipid hydroperoxides are higher, and antioxidant vitamins are lower in individuals who are resistant to insulin-stimulated glucose disposal but otherwise glucose tolerant, nonobese, and normotensive (Facchini et al. 2000b). 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 (Dilman 1994; Facchini et al. 2000a).

The anti-diabetics biguanides inhibit fatty acid oxidation, inhibit gluconeogenesis in the liver, increase the availability of insulin receptors, inhibit monoamine oxidase (Muntoni 1974, 1999), increase sensitivity of hypothalamo-pituitary complex to negative feedback inhibition, reduce excretion of glucocorticoid metabolites and dehydroepiandrosterone-sulfate (Dilman 1994). These drugs have been proposed for the prevention of the age-related increase of cancer and atherosclerosis, and for retardation of the aging process (Dilman 1971, 1994). It has been shown that administration of phenformin into patients with hyperlipidemia lowers the level of blood cholesterol, triglycerides, and β-lipoproteins. It also inhibits the development of atherosclerosis, reduces hyperinsulinemia in men with coronary artery disease. It increases hypothalamo-pituitary sensitivity to inhibition by estrogens and dexamethasone, causes restoration of estrous cycle in persistent-estrous old rats, improves cellular immunity in atherosclerotic and cancer patients, lowers blood somatomedine levels in cancer and atherosclerotic patients with Type IIb hyperlipoproteinemia (Dilman et al. 1978, 1982, 1988; Dilman and Anisimov 1979; Dilman 1994). The available data on effects of antidiabetic biguanides are summarized in the Table 4. Recently it was shown that metformin decreases platelet superoxide anion production in diabetic patients (Gargiulo et al. 2002). It is worth noting that phenformin can

Table 4. Effects of antidabetic biguanides (Muntoni 1977, 1999; Dilman 1994).

Effects on metabolic parameters
Reduction of excessive insulin levels after glucose loading in obese and diabetic subjects
A decrease in IGF-1 in the serum
Inhibition of hepatic gluconeogenesis
Lowering of blood cholesterol and triglyceride levels
An increase in the insulin sensitivity of target cells
An increase in glucose utilization
A decrease in cholesterol metabolism in human fibroblasts
A decrease in the concentration of cholesterol in lymphocytes and platelets
Effects on homeostatic regulation
An increase in the sensitivity of the hypothalamo-pituitary complex to inhibition by estrogens
A restoration of ovarian function in aged rats
An increase in the sensitivity of the hypothalamo-pituitary complex to inhibition by glucocorticoids
An altered hypothalamic regulation of ingestive behaviors
Increase dopamine release in mesolimbic structures
A lowering of monoamine oxidase activity
Effects on age-related pathology
Inhibition of atherosclerosis
Inhibition of spontaneous tumors development
Reduction in blood pressure in hypertensive patients
An increase in blood fibrinolytic activity
An improvement in indices of cellular immunity in humans
A decrease in the immunosuppressive effect of a potent carcinogen, 1,2-dimethylhydrazine

modulate neuronal responses to glutamate and can protect neurons against excitotoxicity by a mechanism involving cellular calcium homeostasis (Lee et al. 2002). The authors suggest possible use of antidiabetic biguanides in the prevention and/or treatment of neurodegenerative processes.

Many characteristics of mice that are long lived due to genetic modifications resemble effects of caloric restriction in wild-type (normal) animals (Table 5). At least three genes (*Pit1*^{dw}, *Prop1*^{dw}, *Ghr*) has been identified in which knockout lead to dwarfism with reduced levels of IGF-1 and insulin and to increased longevity (Flurkey et al. 2001; Coschigano et al. 2000). In Ames and Snell dwarf mice, sexual maturation is delayed, and only few males are fertile, while females are sterile (Bartke and Turyn 2001). These mice as well as *Ghr*$^{-/-}$ knockout mice have significantly reduced glucose levels and fasting insulin levels, decreased tolerance to glucose and increased sensitivity to insulin which appears to be combined with reduced ability to release glucose in response to acute challenge (Bartke and Turyn 2001; Bartke et al. 2003).

New evidence on critical insulin/IGF-1 signaling pathway in the control of mammalian aging and of the involvement of this pathway in longevity of IGF-1 deficient mice were provided recently by Hsieh et al. (2002a, b). It was shown that in the Snell dwarf mice, GH deficiency would lead to reduced insulin secretion and alterations in insulin signaling via InRβ, IRS-1 or IRS-2 and P13K affects genes involved in the control longevity. The authors concluded that the *Pit1* mutation may result in physiological homeostasis that favors longevity.

It was shown that *Igf1r*$^{+/-}$ mice with the *Igf1r* gene inactivated by homologous recombination live on average 26% longer than their wild-type littermates (Holzenberger et al. 2003). These long-lived mice do not develop dwarfism, their energy metabolism was normal. Food intake, physical activity, fertility and reproduction were also unaffected in *Igf1r*$^{+/-}$ mice and embryonic fibroblasts derived from them, were more resistant to oxidative stress than controls. The extension of longevity was also observed in fat-specific insulin receptor knockout (FIRKO) mice (Bluher et al. 2003). These animals have reduced fat

Table 5. Comparison of characteristics of rodents subjected to normal aging, caloric restriction, genetic modifications or treatment with antidiabetic biguanides.

	Tolerance to glucose	Sensitivity to insulin	Serum level			Body weight	Reproductive function	Resistance to oxidative stress	Tumor incidence	References
			Insulin	GH	IGF-1					
Aging	↓	↓	↑	↓	↓	↑	↓	↓	↑	Weindruch and Sohal (1997)
Caloric restriction	↑	↑	↓	↓	↓	↓[a]	↓[d]	↑	↓	Mattson et al. (2003)
Dwarf mice	↓	↑	↓	Absent	↓	↓	↓[d]	↓	= or ↓	Bartke and Turyn (2001)
$GHR^{-/-}$ mice	↓	↑	↓	↑[b]	↓	↓	↓[d]	↓	=	Bartke and Turyn (2001)
$IGF\text{-}1^{+/-}$ mice	↓↑[c]	↑	=	ND	↓	↓	=[d]	↑	=	Holzenberger et al. (2003)
FIRKO mice	= or ↑	↓ in fat	↓	↓	↓	↓[a]	ND	↑	ND	Bluher et al. (2003)
Antidiabetic biguanides	↑	↑	↓	↓	↓	↓	↑[d]	↑	↓	Anisimov (1987)

[a]Percent of body fat also decreased.
[b]The elevated of GH levels in these mice does not imply increased GH signaling because GH receptors are absent in $GHR^{-/-}$ mice.
[c]The tolerance to glucose is increased in females but decreased in male.
[d]Reproductive function in relation to normal aging mice.

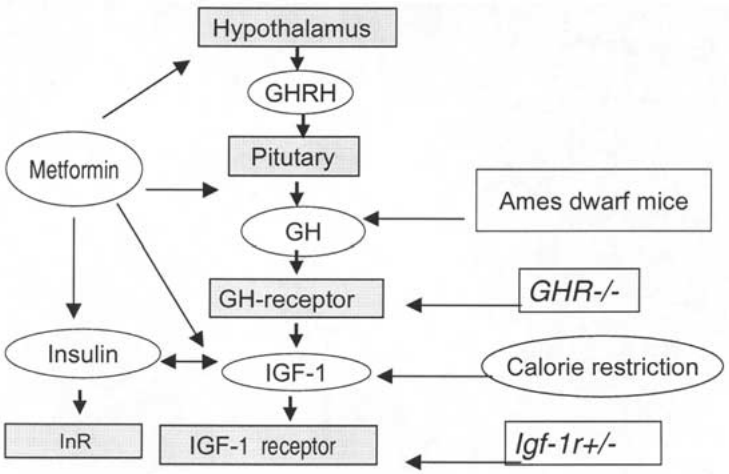

Figure 4. Proposed effects of metformin, calorie restriction and genetic modifications on insulin/IGF-1 signaling pathway.

mass and were protected against age-related obesity and its subsequent metabolic abnormalities including deterioration in glucose tolerance, although their food intake was normal. Both male and female FIRKO mice were found to have an increase in mean life span (by 18%) with parallel increases in maximum life span. The authors believe that decreased fat mass could lead to a decrease in oxidative stress in FIRKO mice. Another possibility is that the increased longevity in these mice is the direct result of altered insulin signaling.

Reduction in both glucose and insulin levels as well as an increase in the sensitivity to insulin are a well-documented response to caloric restriction in rodents and monkey (Weindruch and Sohal 1997; Roth et al. 1999, 2003). It was shown that improved sensitivity to insulin in caloric restricted animals is specifically related to reducing visceral fat (Barzilai and Gupta 1999). It is worthy to note that $Ghr^{-/-}$ mice have a major increase in the level of insulin receptors (Dominici et al. 2000), while Ames dwarf mice have a smaller increase in insulin receptor and substantially increased amount of insulin receptor substrates IRS-1 and IRS-2 (Dominici et al. 2002). The development of tumors in Ames dwarf mice was postponed and the incidence was reduced as compared to the control (Ikeno et al. 2003).

Comparison of characteristics exposed to these endogenous and exogenous influences shows a number of similarities but also some differences (Figure 4). Effects of antidiabetic biguanides seems to be more adequate in the prevention of age-related deteriorations in glucose metabolism and in insulin signaling pathway as well as in important longevity para-

meters such as a fertility and a resistance to oxidative stress and tumorigenesis than those induced by caloric restriction and genetic manipulations.

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