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Metformin

Do we finally have an anti-aging drug?

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Studies in mammals have demonstrated that hyperglycemia and hyperinsulinemia are important factors in aging and cancer. Inactivation of insulin/insulin-like signaling increases lifespan in nematodes, fruit flies, and mice. Life-prolonging effects of caloric restriction are in part due to reduction in IGF-1, insulin, and glucose levels. Antidiabetic biguanides such as metformin, which reduce hyperglycemia and hyperinsulinemia by decreasing insulin resistance, extend lifespan, and inhibit carcinogenesis in rodents. Will antidiabetic biguanides increase lifespan in humans?

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During the last decade it was established that both the insulin/IGF-like signaling (IIS) and nutrient response pathways defined by the mechanistic target of rapamycin (mTOR) pathways control aging and age-associated pathology in yeast, worms, insects, and mammals.[1-3] In each of these organisms, genetic downregulation of the TOR (target of rapamycin) pathway can lead to major extension of longevity. 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 to maintain youthful levels of function and vitality in laboratory mammals at advanced chronological age.[4] Studies in CR rhesus monkeys have produced physiological responses strikingly similar to those observed in rodents and delayed the onset of age-related diseases, but effects on longevity were not consistent.[5] Data from these studies indicate that long-term CR reduces morbidity and mortality in primates, and thus may exert beneficial "anti-aging" effects in humans. 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 to extending human healthspan and lifespan.[3]

The crucial event of the effect of CR is low levels of insulin and insulin-like growth factor-1 (IGF-1) and also an increase insulin sensitivity in rodents[6] as well as in monkeys.[7] 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.[8-10] Whole-genome analysis of gene expression during aging of nematode worm *C. elegans* provided a new evidence on the role of insulin homolog genes and *SIR2* homologs in longevity by interacting with the *daf-2/age-1* insulin-like signaling pathway and regulating downstream targets.[11] 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.[12]

Hyperglycemia is an important aging factor involved in generation of advanced glycosylation end products (AGEs).[13-15] Untreated diabetics with elevated glucose levels suffer many manifestations of accelerated aging, such as impaired wound healing, cataracts, vascular and microvascular damage.[16] The accumulation of the AGE, pentosidine, is accelerated in diabetics and has been suggested to be a reliable biomarker of aging.[13,17] 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 development of cancer.[16,18-20]

Distubances in insulin signaling and carbohydrate homeostasis in diabetes produce 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 biguanides (phenoformin, buformin, and metformin) in premature aging and cancer prevention is of considerable scientific, clinical, and public-health interest. The concept of CR mimetics is now being intensively explored.[21-23] CR mimetics involves interventions that produce physiological and anti-aging effects similar to CR. In comments published by *Science*, 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".[24] There is an exponential growth of publications on reduced risk of cancer and cardiovascular diseases in patients treated with metformin.[3,25-28]

Antidiabetic biguanides seem to be more effective than caloric restriction and some of the genetic manipulations in preventing age-related deterioration of insulin levels.[3] It remains to be shown whether antidiabetic biguanides extend lifespan independently of calorie restriction (CR).[4] Recently, it was published that metformin improves healthspan and lifespan in mice.[29] It worthy to note that Vladimir M Dilman was the first who suggested the use of biguanide antidiabetic drugs as a potential anti-aging treatment.[16,30]

The antidiabetic drugs phenformin (1-phenylethylbiguanide), buformin (1-butylbiguanide hydrochloride), and metformin (N,N-dimethylbiguanide) were shown 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.[16,18,30,31] Currently, phenformin is not used in clinical practice due to it side effects (mainly lactic acidosis) observed in patients with non-compensated diabetes. However there were no cases of lactic acidosis or any other side effects of phenformin given to patients without advanced diabetes.[26,32-34]

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 links between insulin and longevity. In this paper we critically rewieved available data on effects of antidiabetic biguanides on lifespan of worms, flies, mice, and rats.

Effect of Antidiabetic Biguanides on Lifespan in Worms

There are 3 reports on the effects of the biguanides on lifespan in worms. Buformin supplemented nutrient medium in various concentrations (from 1.0 to 0.000 01 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 with the controls.[35] 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.[36] These authors have shown that metformin prolongs nematode health span, slows lipofuscin accumulation, extends mean lifespan, and prolongs youthful locomotor ability in a dose-dependent manner.[36]

In a most comprehensive, elegant study, Cabreiro et al.[37] have shown that both, metformin and phenformin decelerate aging in *C. elegans* in dose-dependent manner. Metformin at doses 25, 50, and 100 mM increased mean lifespan by 18%, 36%, and 3%. Phenformin at 1.5, 3, and 4.5 mM increased lifespan by 5%, 21%, and 26%. The authors revealed that metformin increases lifespan by altering microbial folate and methionin metabolism.

Effect of Antidiabetic Biguanides on Lifespan in *Drosophila*

Metformin given in doses 0.4, 0.8, or 0.16 mg/ml did not influence the mortality rate in *Drosophila*.[38] Metformin treatment had no effect on survival of male flies maintained on food containing from 1 to 50 mM metformin, with a significant decrease in survival at 100 mM metformin.[39] In female *Drosophila*, metformin given in doses from 1 mM to 10 nM did not affect lifespan, while doses above 10 mM resulted in a dose-dependent decrease in lifespan. The authors showed that feeding metformn to adult flies resulted in a robust activation of AMPK and reduced lipid store. Analysis of intestinal physiology after treatment with metformin suggests that theses effects may depends on disruption of intestinal fluid homeostasis.[39] It was shown that metformin reduces age- and oxidative stress-related accumulation of DNA damage marked by *Drosophila* γH2AX foci and 8-oxo-dG in intestinal stem cells and progenitor cells derived from midgut.[40] Metformin also in suppressed age- and oxidative stress-related hyperproliferation of intestinal stem cells as well as intestinal hyperplasia. These findings suggest a possible impact of DNA damage on stem cell genomic instability, which leads to the development of age-related changes.

Effect of Antidiabetic Biguanides on Aging and Lifespan in Mice

The results of studies on effect of antidiabetic biguanides on lifespan in mice and rats are summarized in **Table 1**. Female C3H/Sn mice were fed a standard diet ad libitum and were given phenformin orally at a single dose of 2 mg/mouse/d until a natural death.[41,42] The treatment with phenformin prolonged the

Table 1. Effects of antidiabetic biguanides on lifespan and spontaneous tumor incidence in rodents

Strain, species	Sex	No. of mice, C/T[1]	Age at start of treatment, months	Drug[2]	Dose and route of treatment[3]	Effect on mean lifespan, %	Effect on tumor incidence[4]	References
C3H/Sn mice	F	30/24	3.5	PF	2 mg/mouse p.o.	+21%	↓	41
HER-2/neu mice	F	34/32	2	MF	100 mg/kg, d.w.	+8%	↓	43
HER-2/neu mice	F	31/35	2	MF	↑	+4%	↓	44
SHR mice	F	50/50	3	MF	↑	+38%	=	45
SHR mice	F	119/51	3	MF	↑	+14%	=	46
		97/45	9	MF	↑	+6%	=	
		69/33	15	MF	↑	0	=	
129/Sv mice	F	47/41	3	MF	↑	+5%	↓	47
129/Sv mice	M	41/46	3	MF	↑	-13%	=	
C57BL/6 mice	M	64/83	12	MF	0.1% in diet	+5.83%	=	29
	M	90/88	12	MF	1% in diet	-14.4%	↓	
B6C3F1 mice	M	297/36	12	MF	0.1% in diet	+5.83%	=	
LIO rats	F	41/44	3.5	PF	5 mg/rat, p.o.	0	↓	43 and 50
LIO rats	F	74/42	3.5	BF	↑	+7%	↓	
F344 rats	M	31/40	6	MF	300 mg/kg, b.w.,	0	N.D.	53

Notes: [1]C/T, control/treatment; [2]BF, buformin; MF, metformin; PH, phenformin;[3] d.w., drinking water; p.o., per os;[4] ↑, increases; ↓, decreases; = , no effect; N.D., not detected.

mean lifespan of female these mammary cancer-prone mice by 21% ($P < 0.05$) and the maximum lifespan by (26%) in comparison with the controls. At the time of death of the last mice in the control group 42% of phenformin-treated mice were alive.

Exposure to metformin did not change the body weight or temperature, slowed down the age-related rise in blood glucose and triglyceride levels, reduced the serum level of cholesterol and β-lipoproteins, delayed the age-related irregularity in estrous cycle, extended the mean lifespan by 4–8%, and the maximum lifespan by 1 mo in transgenic HER-2/neu mice in comparison with the control animals.[43,44] The metformin treatment normalized the expression of cytolytic granzyme B and perforine genes in mammary carcinomas in these mice.[43]

The treatment of female SHR mice with metformin increased the mean lifespan of the last 10% of survivors by 20.8% and maximum lifespan by 2.8 mo (10.3%) in comparison with the control mice.[45] It was observed that metformin decreased body temperature and postponed age-related switching-off of estrous function. Metformin did not affect serum level of cholesterol, triglycerides, glucose, and insulin and failed to influence spontaneous tumor incidence in these animals. In another set of experiments, female SHR mice were given meformin at the same dose starting from the age 3, 9, or 15 mo.[46] Administration of metformin started at the age of 3 mo increased mean lifespan by 14% and maximum lifespan by 1 mo, whereas the treatment started at the age of 9 mo, by only 6%, and started at the age of 15 mo failed to influence.

The long-term treatment of inbred 129/Sv mice with metformin (100 mg/kg in drinking water) slightly modified the food consumption but failed to influence the dynamics of body weight, decreased by 13.4% the mean lifespan of male mice, and slightly increased the mean lifespan of female mice (by 4.4%). The treatment with metformin failed to influence spontaneous tumor incidence in male 129/Sv mice, decreased by 3.5 times the incidence of malignant neoplasms in female mice, while somewhat stimulated formation of benign vascular tumors in the latter.[47]

Metformin treatment significantly prolonged (by 20.1%) the survival time of male (but not female) transgenic mice with Huntington disease (HD) without affecting fasting blood glucose levels. There was no increase in survival of mice with the increase of the dose of the drug.[48]

In recent paper by Martin-Montalvo et al.,[29] male C57BL/6 mice were given ad libitum diet with supplementation of 0.1% or 1% of metformin starting from the age 54 weeks for the remainder of their lives. The mean lifespan of mice treated with 0.1% metformin diet was increased by 5.83% as compared with the relevant control group of mice, whereas the dose 1% was toxic and reduced the mean lifespan by 14.4%. Diet supplementation with 0.1% metformin increased lifespan by 4.15% in another strain of mice, B6C3F1. No numerical data on maximal lifespan of mice of any group were presented in the paper. It was observed that C57BL/6 mice were lighter than those in control group between the age of 72 to 90 wk and were heavier them by the age of 124 wk. These effects were not observed in male B6C3F1 mice. There were no significant differences in pathologies observed in both strains of mice fed diet with 0.1% metformin. However, as it could be calculated from the data presented in Martin-Montalvo et al., Table S1, diet with 1% metformin led to significant reduction of liver cancers incidence (3.3% in the metformin group and 26.5% in control group, $P < 0.001$).

Table 2. Changes developing in organism during natural aging and carcinogenesis:effects of antidiabetic biguanides (for reference, see 3, 51, and 69)

Parameters	Aging	Carcinogeness	Biguanides
Molecular level			
Free radical generation	↑	↑	↓
AGEs formation	↑	↑	↓
DNA adducts formation	↑	↑	↓
DNA repair efficacy	↓	↓	↑
Genomic instability	↑	↑	↓
Telomerase activity	↓	↑	↓
Telomere lenght	↓	↓	↑
mTOR activity	↑	↑	↓
Clock gene expression (*Per1*, *Per2*)	↓	↓	↓
Mutation rate	↑	↑	↓
Oncogene expression	↑	↑	↓
p53 mutations	↑	↑	?
Cellular/tissue level			
Oxidative stress	↑	↑	↓
Chromosome aberrations	↑	↑	↓
Induced pluripotent stem cells (iPSC)	↓	↓	↑
Proliferative activity	↓	↑	↓
Focal hyperplasia	↑	↑	↓
Apoptosis	↓	↓	↑
Autophagy	↓	↓	↑
Angiogenesis	↓	↓	↓
Cell-to-cell communication	↓	↓	↑
Senescent cells number	↑	↑	↓
Latent (dormant) tumor cells number	↑	↑	↓
Systemic/organism level			
Hypothalamic threshold of senisitivity to homeostatic inhibition by steroids	↑	↓	↓
Tolerance to glucose	↓	↓	↑
Serum insulin level	↑	↑	↓
Susceptibility to insulin	↓	↓	↑
LDL and cholesterol level	↑	↑	↓
Ovulatory function	↓	↓	↑
Fertility	↓	↓	↑
T-cell immunity	↓	↓	↑
Inflammation	↑	↑	↓
Cancer risk	↑	↑	↓
Lifespan	↓	↓	↑

Notes: ↑, increases; ↓, decreases; ?, no data.

Male 57BL/6 mice given metformin had lower rates of cataracts.

The authors stressed that treatment with metformin mimics some of the benefits of calorie restriction, such as improved physical performance, and prevented the onset of metabolic syndrome: improved glucose-tolerance test, increased insulin sensitivity, and reduced low-density lipoprotein and cholesterol levels without a decrease in caloric intake. At a molecular level, metformin increases AMP-activated protein kinase activity and increases antioxidant protection, resulting in reductions in both oxidative damage

accumulation and chronic inflammation.[29] Metformin also exerts CR-like genomic and metabolic responses, which were interpreted as induction of associated with longevity pathways in mice.

Effect of Antidiabetic Biguanides on Aging and Lifespan in Rats

We studied the influence of phenformin and buformin on lifespan and spontaneous tumor development in rats. Buformin or phenformin was given beginning from 3.5 mo of age up to the natural death of the animals.[42,49-51] Both drugs reduced the body weight of rats. This effect was not associated with a reduced food intake. The disturbances in estrus function were apparent in 38% of the control rats aged 16 to 18 mo and in only 9% of buformin treated rats of the same age. Buformin caused a 9% increase in the mean lifespan ($P < 0.05$) and a 1.6-fold reduction in cumulative incidence of spontaneous tumor. The number of tumors per animal decreased nearly 2-fold under the influence of buformin. Phenformin did not increase the mean lifespan, but the maximal longevity increased by 3 mo. This was associated with a 1.3-fold decrease in cumulative tumor incidence and a 2-fold decrease in the mean number of tumors per animal.[50,51] These results are in agreement with the observations on absence of carcinogenic effect of phenformin.[52]

In the study of Smith et al.,[53] 6-mo-old male F344 rats were randomized to one of 4 diets: control, calorie restricted (CR), metformin, and pair fed to metformin. The CR group showed significantly reduced body weight and food intake throughout the study. Body weight was significantly reduced 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, metformin-treated, and pair fed F344 rats as compared with control.[53] CR significantly increased lifespan in the 25th quantile but not the 50th, 75th, or 90th quantile. Rats given metformin or the pair feeding were not significantly different from controls at any quantile. The authors suggest that reduced efficacy of CR in this study might provide a partial explanation for the lack of an increase in lifespan with metformin.

Controversies and Future Directions in Research on Antidiabetic Biguanides as Geroprotectors

Thus, available data show that administration of antidiabetic drugs did not influence lifespan in fruit flies and is capable of increasing survival of worms and rodents. This effect varied depending on strain and species of animals. Four strains of female mice and 3 strains of male mice were treated with antidiabetic biguanides (only in one strain both sexes were used). Only single studies were performed with female rats treated with buformin or phenformin and with male rats treated with metformin. The experiments with both male and female animals of different strains need to be performed to conclude on geroprotective potential of antidiabetic biguanides.

Modes of administration and doses of metformin varied. The NIA (National Institute of Aging, NIH) teams used diet supplementation with 0.1% or 1% metformin in males of one strain of mice (C57BL/6) and one diet with 0.1% metfromin in male B6C3F1 mice,[29] whereas the PRIO (Petrov Research Institute of Oncology) team administered metformin with drinking water at a dose 100 mg/kg to female HER-2/neu mice in 2 independent studies[43,44] to outbreed female Swiss-derived SHR mice, also in 2 sets of experiments,[45,46] and in male and female inbred 129/Sv mice in one study.[47] Calculations show that C57BL/6 mice that consumed diet with 0.1% metformin received metformin at a dose ranging from 75–100 mg/kg of the body weight, and B6C3F1 mice at the doses 67–90 mg/kg, practically same level of the doses got with drinking water. The highest dose of metformin was given to male C57BL/6 mice (1% in diet) reduced their mean lifespan by 14.4%, and were nephrotoxic.[29] The toxicity of metfromin was studied in rats.[54] Metformin was given by oral gavages for 13 wk in doses 200, 600, 900, or 12 000 mg/kg/day. Administration of metformin at a dose 900 mg/kg or more resulted in moribundity/mortality and clinical signs of toxicity, whereas no observable adverse effect was seen at 200 mg/kg/d.[54]

The NIA team started treatment of mice at the age of 54 wk (12 mo). The PRIO team started the treatment of mice at the age of 2–3.5 mo in most experiments. In female SHR mice, metformin was given from the age of 3, 9, or 15 mo, resulting in attenuation of effect on lifespan with the increase in the age at start.[46]

On the whole, the data in the literature and the results of our experiments suggest that antidiabetic biguanides are promising for lifespan extension. Further studies are needed to determine the doses and the age of the onset of administration of metformin for premature aging in humans.

Conclusion

During the last decade, the intensive search of anti-aging remedies has lead to the conclusion that both the insulin/IGF-like signaling and nutrient response pathways such as the mechanistic target of rapamycin (MTOR) control aging and age-associated pathology in yeast, worms, insects, and mammals.[1-3,55] mTOR is activated by nutrients, cytokines, insulin, and related growth factors through phosphatidylinositol-3-OH kinase (PI3K) and AKT kinase signaling and suppressed by AMP-activated protein kinase (AMPK), a key sensor of cellular energy status. mTOR stimulates protein and lipid biosynthesis, inhibits autophagy, and regulates mitochondrial function and glucose metabolism. Also, mTOR drives geroconversion from cell cycle arrest to senescence[56-59] and is involved in organismal aging.[60,61] Genetic data suggest the metformin acts through a similar mechanism. Energy sensor AMPK and AMPK-activating kinase LKB1, which are activated in mammals by metformin treatment,[56,57] are essential for health benefits in *C. elegans,* suggesting that metformin engages a metabolic loop conserved across phyla.[36] It was also shown that metformin activated SKN-1/Nrf2, oxidative stress-responsive transcription factor.[58] Effects of biguanides on the senescence-associated secretory phenotype interfered with IKK-β/NFκB—an important step in hypothalamic programming of systemic aging.[1,59,62-65]

There are 9 tentative hallmarks of aging in mammals, which may represent common denominators of aging in different organisms: genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered cell-to-cell communication.[55] There is also the sufficient similarity in patterns of changes observed during normal aging and the process of carcinogenesis. This aspect has been discussed in detail in our works since 1983.[51,66-69] Metformin seems to influence all of them. Data on physiological and molecular mechanisms of the inhibitory effect of biguanides on lifespan and carcinogenesis have been discussed in several recent papers[1,3,27-29] and summarized in the **Table 2**. All these finding convince us that metformin is promising drug for aging prevention in humans.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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