

cumulative incidence of spontaneous tumors was decreased by 1.6 times in buformin-treated rats as compared with the control rats and the multiplicity of spontaneous neoplasms was decreased almost 2-fold.

Phenformin was given to female outbred LIO rats intragastrally 5 times a week starting from the age of 3.5 months in a single dose of 5 mg rat<sup>-1</sup> day until natural death.<sup>19,20</sup> Administration of phenformin failed to influence the mean life span in rats. At the same time, the mean life span of the last 10% survivors was increased by 10% ( $p < 0.05$ ), and maximum life span was 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 ( $p > 0.05$ ). Disturbances in the estrous function were 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 incidence of spontaneous tumors was decreased by 1.3 times in phenformin-treated rats as compared with the control group.<sup>19,20</sup>

Six month-old male F344 rats were randomly subdivided into four groups and were maintained on one of four diets: control, calorie restricted (CR), metformin (300 mg kg<sup>-1</sup> day<sup>-1</sup>) and pair-fed to metformin.<sup>28</sup> The CR group had significantly reduced food intake and body weight throughout the study. Body weight was significantly reduced in the metformin group compared with the control group during the middle of the study, despite the similar weekly food intake. There were no significant differences in the mean life span or the mean life span of the last surviving 10% of each group in the CR, metformin and pair-fed groups compared with the control. However, the aging rate estimate ( $\alpha$  – slope, rate of increase of mortality) of the Gompertz model in 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 life span in the 25th quantile but not the 50th, 75th, or 90th quantiles. The survival of rats in groups exposed to metformin or to the pair-feeding were not significantly different from the controls at any quantile.<sup>28</sup> The authors stressed the one limitation of this study—the lack of a robust CR response for extension of maximum life span, which has been observed in another CR study using the same rat strain.<sup>31</sup> The reduced efficacy of CR in this study might provide a partial explanation for the lack of a significant increase 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 was approximately 10 times greater than the highest dose used in human treatments, implying that any increase necessary to observe life span benefits is questionable for a human application.<sup>28</sup>

Thus, the available data on the effects of biguanides in rats are very scarce. The early NCI study was terminated before the natural death of the majority of animals. Moreover, the sample sizes (15 rats in the control group) were too small in this study. In our long-term studies, buformin and phenformin were tested only in females and in a single dose, whereas metformin was tested only in male rats and also in a single dose.