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# A glucose-rich diet shortens longevity of mice

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**Abstract**

High plasma levels of glucose and insulin over long time periods play an important role in the genesis of diabetic complications. There is evidence that the long term consumption of glucose-rich diet by rats is detrimental to insulin sensitivity. We investigated the effect of a glucose-rich diet on longevity of 70 female mice which were compared to 70 mice on a control diet. The average age of death of the control group was $568 \pm 139$ days compared to $511 \pm 170$ for the glucose group and the seven oldest mice of the control group died at age $890 \pm 52$ days, while the seven oldest mice of the glucose group died at $833 \pm 49$ days. These differences are statistically significant ($P \leq 0.05$). Our work shows that a life-long intake of a diet with 20% of total energy derived from glucose leads to a significant reduction of the average and maximal life-span in female mice and thus, supports previous observations of detrimental effects of high glucose intake over long periods. Copyright © 1996 Elsevier Science Ireland Ltd.

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## 1. Introduction

It is generally accepted that high levels of dietary fat intake over a life time combined with high levels of plasma lipids have atherogenic consequences and shorten life span. Also, an abnormally high blood glucose level as observed in diabetes mellitus is known to be responsible for severe and permanent damages in many tissues as a series of experimental and clinical observations have proven. A growing body of evidence implies that certain insulin-independent metabolic pathways for glucose lead to products that may play a role in the genesis of diabetic complications and a strict blood sugar control in diabetes mellitus has proven to reduce the incidence and extent of complications frequently occurring in the course of this disease [1]. Especially nonenzymatic glycation processes and their connections to free radical generation may play an important role in the development of pathological lesions [2].

The purpose of the investigation presented here was to evaluate the effect on the longevity of female mice of the addition of glucose to a control diet over life time. To our knowledge no such study has been carried out previously.

## 2. Methods

### 2.1. Animals

The experiments were approved by the University of Graz Animal Experimentation Ethics Committee.

Female Swiss-albino mice (Him OF1)—5 weeks old—were purchased from Forschungsinstitut für Versuchstierzucht und Versuchstierhaltung of the University of Vienna (Himberg, Austria). This mouse stock was originally developed at Carworth, New City, New York, as CF1 and brought to Iffa-Credo L'Arbresle, France, in 1966. The colony at the University of Vienna was developed from mice obtained from Iffa-Credo in 1980 and is maintained under SPF conditions. Mice were housed in the following conditions: temperature, $22 \pm 1$°C; humidity, $55 \pm 10$% and controlled light, 05:30–16:30, in a room with a ventilation system. The mice were maintained in polycarbonate cages with a bottom area of 1820 $cm^2$ in groups of five, thereby avoiding the stress of individual housing, on autoclaved softwood granules. The cages were covered with metal grids. No other animals were present in this facility. The mice always had free access to tap water and food. During the first 2 weeks at the laboratory all mice were fed a commercial cereal based diet (control diet). At 8 weeks of age, weighing $24.0 \pm 1.0$ g they were randomly assigned to two groups: a control group of 70 mice receiving the control diet and a glucose group of 70 mice receiving the control diet plus glucose. The mice were weighed weekly by a veterinarian rodent expert and on these occasions, their health was also checked. None of the mice were subjected to any experimental treatment and they were allowed to die of natural causes. All dead mice were dissected, the organs (liver, spleen, kidneys) were weighed and visually

inspected for tumors, no histological examinations were carried out. Cages were monitored daily for deaths.

### 2.2. Diet

The control diet was a commercial, non purified cereal-based pelleted preparation, supplemented with 1% vitamin premix (T 779 Tagger Kraftfutterwerk, Graz, Austria). The diet contained an average of 223 g protein, 41 g fat and 398 g carbohydrates per kg with a metabolizable energy content of about 12.6 MJ. The glucose diet consisted of control diet pellets and pure glucose pellets: 18.5 g glucose pellets (corresponding to 315 kJ) were offered per 100 g of consumed control diet pellets (corresponding to 1260 kJ). The glucose pellets were bought from a commercial source (Dextro Energen, C.H. Knorr Ges.mbH; Wels/Austria) and were fortified per 100 g with the following vitamins: ascorbic acid 15.2 mg, niacin 3.0 mg, pyridoxin 0.30 mg, riboflavin 0.29 mg, thiamin 0.21 mg. After the remains of the control diet pellets of the previous day had been removed, the mice of the glucose group got at 07:30 h the daily allowance of glucose pellets calculated from the food consumption of the control group. The glucose was offered in five identical pieces and attention was paid to the fact that each of the five animals ate up its allowance. After the palatable glucose pellets had been eaten up during the morning the control pellets were given. Food consumption was calculated daily on a per-cage basis. Spillage was negligible.

### 2.3. Statistical analysis

The cumulative survival probabilities for glucose-fed animals versus controls were estimated by the product limit technique [3]. Statistical differences between these survival curves were tested for significance using the generalized Savage test (Mantel-Cox test) which is known to be particulary sensitive to 'late' differences between survival curves. Additionally, the survival data were analyzed by the proportional hazards technique [4] in order to quantitate the effect of glucose on survival. Differences between mean body weights of the two groups of mice were tested for significance by analysis of variance (ANOVA) for repeated measurements. At each time point $t$ ($t = 4$, 8, 12,... weeks) an ANOVA was performed testing retrospectively the difference of the body weight curves, starting at time = 4 weeks up to time $t$. This type of analysis was performed to account for the statistical dependence of repeated measurements as well as for the dynamic evolution of the body weight curves. The same type of statistical analyses was applied to the data on food consumption.

The statistical analyses were performed using the BMDP software (BMDP Statistical Software, Cork, Ireland). Procedures BMDP2V (ANOVA for repeated measurements), BMDP1L (product-limit method) and BMDP2L (Cox technique) were employed.

## 3. Results

### 3.1. Food consumption

The food consumption per animal as calculated from the food consumption per cage was highest at the beginning of the experiment with an energy intake of about 483 kJ/week in week 8. In week 50, a time point when on the one hand the rapid growth of the animals had leveled off, and on the other hand physical activity like climbing on the cover grid had declined, only about 357 kJ/week were consumed.

No significant differences between the food consumption curves of the two groups over the whole life span were observed ($P \geq 0.10$; ANOVA for repeated measurements). So, for example, the average weekly food consumption between week 50 and 80 was 28 g per week corresponding to 353 kJ in the control group; in the glucose group the animals ate about 24.2 g control diet (corresponding to 305 kJ) and 4.5 g of glucose (corresponding to 76 kJ) summing up also to 381 kJ, a non significant difference of only 28 kJ/week. As the glucose group animals consumed 80% of their energy intake in the form of the control diet and as the glucose pellets were fortified with vitamins no state of deficiency in respect to any essential food components was given. Total energy intakes of the two groups are shown in Fig. 1.

### 3.2. Body weight development

The statistical analysis indicated no significant effect of diet on body weight ($P \geq 0.10$; ANOVA for repeated measurements). Both dietary groups rapidly

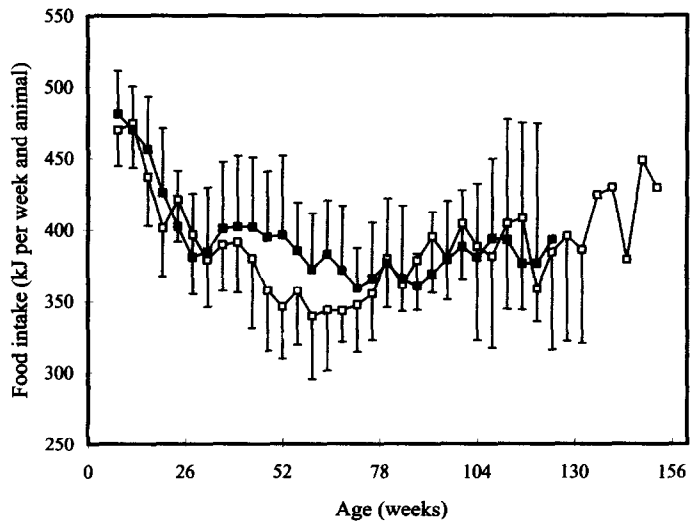


Fig. 1. Average food intake of glucose-rich diet fed mice (■) and the control-diet fed mice (□). Vertical bars indicate S.E. values.

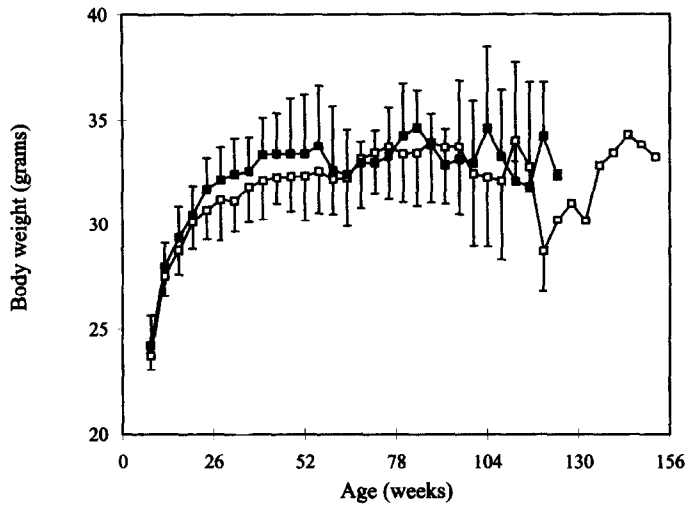


Fig. 2. Average body weights of glucose-rich diet fed mice (■) and the control-diet fed mice (□). Vertical bars indicate S.E. values.

gained weight to about the 24th week and then slowly increased their body mass, attaining a steady state at approximately the 50th week with a mean body weight of about 34 g. Fig. 2 shows the body weight curves. The weights of kidney, spleen and liver were in the normal range and no differences between the two groups were found (data not shown).

### 3.3. Survival analyses

Fig. 3 shows the survival curves of the two groups. We found a significant difference between the two groups which was reflected in different mean, median and maximum life span. The average age of death in the control group was $568 \pm 139$ days, a range also reported by others [5] and in the glucose group $511 \pm 170$ days. The median (the time point when 50% of the animals have died) was in the control group 553 days and in the glucose group 497 days. The maximal life span as seen in the average age of the seven oldest mice was in the control group $890 \pm 52$ (range 805–1078) compared to $833 \pm 49$ (range 763–896) in the glucose group. All these differences were significant: $P = 0.032$ for the average age and $P = 0.056$ for the average age of the six oldest mice. Analysis of the cumulative survival curves by the product limit approach ($P = 0.074$, generalized Wilcoxon test) underscored these conclusions. From the Cox regression the effect of the glucose diet on longevity could be estimated: risk increase of death for a glucose diet animal as compared to a control diet animal (100% relative risk) is 138%, as was calculated from the regression coefficient of 0.3274 (S.E. = 0.1782).

### 3.4. Mortality

The total incidence of visible tumors was in the control group, and in the glucose group the figure was approximately 25%, as normally found in this mouse strain [6]. The tumors were mainly found in the abdominal region of the mamma and on the hind limbs.

## 4. Discussion

The main purpose of this study was to investigate how the addition of glucose in an amount of 20% of total energy intake to a control diet effects the life span of female mice. In order to avoid any possible influence on this parameter, the animals were not subjected to any experimental treatment, except for the weekly weighing procedure.

In accordance with previous work [7,8], we found that mice have a pronounced ability to adjust their food intake according to the density of their diets. So mice consuming 20% of their energy requirement in the form of glucose pellets only slightly exceeded the energy intake of the control group. This ability is especially distinct in mice of the Swiss-albino stock, with their high physical activity, if the animals have the opportunity for voluntary exercise. It is not so pronounced if physical activity is restricted [9]. In humans also, similar results suggest that physical activity plays a pivotal role in energy regulation [10].

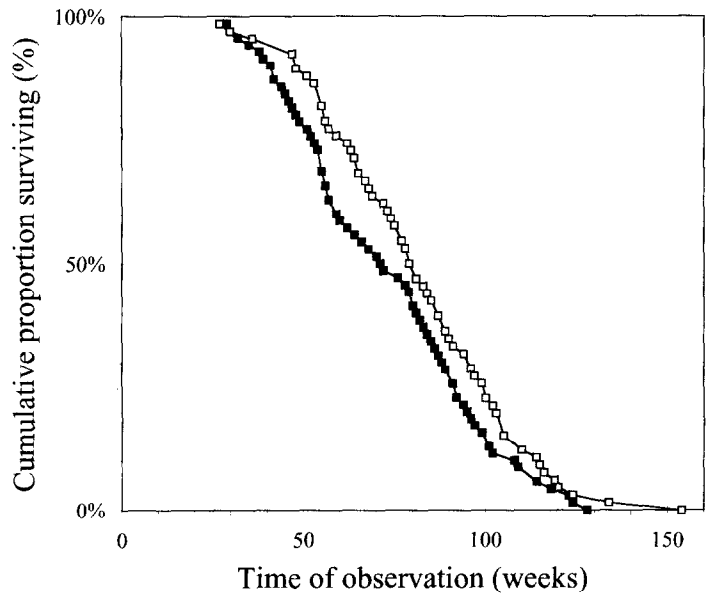


Fig. 3. Cumulative survival curves of glucose-rich diet fed mice (■) and the control-diet fed mice (□), obtained by the product-limit method.

As a consequence of the same energy intake, body weight development and final body weight were not significantly altered by the diets and so the energy efficiency (gram body weight gain per consumed joule) was not different between the two groups and both thrived equally well.

Masoro et al [11] explored in a life span longitudinal study the diurnal pattern of plasma glucose concentration in male F344 rats with free access to food. They found a minimum in the glucose concentration contours during the morning hours, a time period when the rats would not eat the normal diet. We offered at 07:30 the glucose pellets which were very palatable and were eaten up by the animals until about 1100. Each animal consumed approximately 640 mg of glucose daily after week 50 of life, having reached the final body weight of about 34 g. Such an amount of glucose with its high glycemic response leads postprandially to an elevated plasma glucose level over these hours. If mice show a similar circadian rhythm in the glucose curve as the rat, a relatively high glucose blood concentration caused firstly by the intake of glucose and secondly by the intake of food should be maintained over nearly 24 h and should lead to a state of 'glycative stress'. Masoro et al. [11,12] also showed that the increase in longevity by dietary restriction was connected with lowered plasma glucose and insulin levels throughout the life span and they suggested that this decrease could be the reason for the retardation of the aging process by dietary restriction. In our experiment the probable lifelong elevation of the levels of blood glucose and insulin, firstly by the free access to food and secondly by the intake of glucose, could be the cause of the opposite effect, namely, a reduction in life span of about 10%.

There has been a long controversy in the treatment of diabetes as to whether strict control of blood sugar has a beneficial effect. Accordingly, dietary advice as to whether the carbohydrate or the fat content should be high or low varied. However, experimental and clinical observations over the past 30 years have yielded convincing evidence that chronic hyperglycemia is linked with micro- and macrovascular disease, with impaired cellular immunity and with cell cycle abnormalities [13]. Additionally, hyperinsulinemia is implicated in coronary heart disease [14], hypertension [15] and arteriosclerosis [16]. The functional and morphological damage seems to be not only a consequence of high postprandial peak concentrations of plasma glucose and insulin but mainly the consequence of a persistent chronic state of hyperglycemia combined with hyperinsulinemia. Based on this knowledge it has been suggested that the dietary recommendation concerning blood glucose control be based on a 'glycemic index' [17], a value that compares the area under the blood glucose response curve for a test food with that of glucose giving the highest response.

Conditions of hyperglycemia and hyperinsulinemia when acting over a life span could be part of the aging process as Cerami [18] suggested.

A reduction of life span in rats by about 10% was also found by Murtagh-Mark [19] in a study on the effect of sucrose compared with starch feeding. They speculated that the underlying mechanism for the different effects of dietary sucrose and starch may be differences in the interaction between advanced glycation and oxidative stress. This speculation is based on studies [20] which show that pento-

sidine, an advanced glycation product, was decreased in energy-restricted sucrose fed rats but not in energy-restricted corn starch-fed rats. These findings indicate that various dietary carbohydrates may cause glyco-oxidative damage to different extents. This suggestion is consistent with recent reviews [2,21,22] describing the many pathways between non-enzymatic glycation of proteins and lipoproteins and free radical generation mediated by glucose autoxidation [23] affecting virtually every aspect of homeostasis. They suggested that the fructose component of sucrose may be responsible for the described effects. Our results show that also glucose seems to be a candidate. In a very recent investigation comparing different dietary carbohydrate sources Higgins et al. [24] found that the more rapidly dietary carbohydrates are absorbed the faster insulin resistance develops and that glucose-fed animals displayed insulin resistance only after 8 weeks of feeding. Ishii et al. [25] provided support for the hypothesis that the activation of an isoform of protein kinase in vascular tissue is a key step through which glucose triggers diabetic complications.

In summary, then, the data presented in this study suggest that the consumption of 20% of total energy in the form of glucose can significantly reduce life span of female Swiss albino mice without influencing total energy intake and growth.

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