Dietary restriction: it’s never too late

Bachelor thesis

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August, 2010

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Abstract

Dietary restriction (DR), the consumption of less food while avoiding malnutrition, increases lifespan in various invertebrate organisms. In the fruit fly lifespan is extended by decreasing the risk of death. Lifespan can also be extended by slowing the rate of aging. The mechanisms by which lifespan is extended in rodents during DR is uncertain. Experiments on rodents lack sufficient sample sizes to draw distinct conclusions from. By studying death rates from several study using the DR regime in mice I can create a larger sample size. Using the Gompertz model I show that the increase in lifespan in mice results primarily from an acute reduction in the risk of death. The current nutritional status determines the chance of survival and hence DR instigated at any time could still increase lifespan. Because rodents share many biological characteristics with humans, these results can possibly be extrapolated to humans.

Key words: Dietary restriction; lifespan; risk of death; rate of aging; Gompertz

1. Introduction

Extending life expectancy is a strong desire of adult human beings. However, aging remains an inevitable characteristic of life. During aging, deleterious changes in the body lead to the physiological decline of functions and accumulation of irreversible damage (Kristal & Yu, 1994). This plays a role in the mechanism of aging. In absence of an effective intervention, the rate of mortality increases as age increases. A useful intervention that is widely studied is dietary restriction (DR). Reduction of nutrient intake has been shown to extend lifespan in various invertebrate organisms, including yeast (*Saccharomyces cerevisiae)* and the fruit fly (*Drosophila)* (Koubava and Guarente, 2003). DR is therefore a candidate for an evolutionary conserved mechanism through which life expectancy can be extended. Interventions that extend maximum-lifespan appear to delay the appearance of pathology and keep organisms in a youthful state for longer (Partridge et al., 2004). Therefore it has been suggested that DR slows down the rate of aging. Analysis of mortality rates can be a test of this hypothesis. Research on mortality rates of *Drosophila* show that the rate of aging remains unaffected when applying the DR regime, although mortality rates are lower during DR in *Drosophila*. Could it be that DR affects another mechanism that extends lifespan? Fully fed *Drosophila* flies at 14 days of adulthood switched to DR showed rapid reduction in mortality (Mair et al., 2003). Within 48 hours of the switch, DR flies showed the same mortality trajectories as same-aged flies maintained on DR throughout adulthood (Mair et al.,2003). Switching DR flies back to full feeding at later ages caused an acute increase in mortality rates within 48 hours. This finding shows that mortality in *Drosophila* depends upon their current nutritional status and that DR has an acute and reversible effect. There is no memory of past feeding (Mair et al., 2003). Increase by DR of *Drosophila* lifespan is solely due to reducing the short-term risk of death and not by slowing the rate of aging. The reduced risk of death by DR in *Drosophila* contrasts sharply with the effect that temperature has on lifespan of *Drosophila* (Mair et al., 2003). Flies switched from the 27 °C environment to the 18 °C environment exhibited a reduction in mortality with age. When the flies were switched back to the 27 °C environment, the rate of increase in mortality with age rose again. Elevated temperature leads to accumulation of a higher level of irreversible damage (Partridge et al., 2005). However, flies switched back to the 27 °C environment had lower mortality rates compared to the flies maintained on 27 °C. Lowered temperature therefore extends lifespan by lowering the rate of

accumulation of irreversible damage. Thermal history in *Drosophila* thus affects the rate of aging in which there is memory of past temperature.

In other words: There are two mechanisms through which lifespan can be extended. Both mechanisms are of importance for further research on DR. The interesting question is whether DR has the ability to extend lifespan of humans. If it helps us to extend our lifespan, in other words lifespan from adulthood, we can use this tool that is easily accessible. However, we have to know which mechanism applies in humans during DR. Only then we can start to look how DR could be to our own benefit. It makes a difference whether DR decreases our risk of death or rate of aging in when and how we when to apply DR. Research on rodents could help us to find out how DR might works in humans, as these species share many biological characteristics with humans. Finding a larger effect of one mechanism could mean a similar effect of that mechanism in humans.

Even though it is established that DR increases lifespan in rodents, the mechanism by which life extension is extended is still unclear (Koubova et al., 2003). Earlier reports suggest that rats subjected to DR show a lower risk of death and also a decrease in the process of aging (Partridge et al., 2003). Other reports on mice conclude that the extended lifespan by DR is entirely due to a decrease in the risk of death (Weindruch et al., 1986). To distinguish between the two mechanisms by which the DR regime operates, a large number of individuals is required. Large sample sizes are needed to make an accurate comparison between the mechanism in DR and ad libitum fed animals. Sample sizes of earlier studies on rodents may not have been sufficiently large to draw a distinct conclusion. Most of the studies use sample sizes of a maximum of 100 rodents. Studies on *Drosophila* however reach sample sizes ranging from 7000 to 8000 flies. Studying this amount of flies at one moment is attainable, as housing of flies is not difficult and flies are short lived. Using large sample sizes of rodents at one moment is complicated considering the housing and longer study period due to their longer lifespan. This makes research on rodents unpractical. Also the ethical aspect should be kept in mind when implementing an experiment with many rodents. Nevertheless, the importance of an experiment with a larger sample size greater than in previous studies is of great importance. If results on rodents become more clear and distinct due to a larger sample size, extrapolation to humans becomes more relevant. Therefore, in this thesis I will study which mechanism affects mice under DR in relation to extending lifespan using data obtained from the literature. To account for the problem with sample sizes in earlier reports, I will analyze survival curves from 3 published studies using the DR model of retarded aging in mice. By pooling these data I create a larger sample size than current studies. This allows a more accurate comparison between groups. In this way I hope to identify how DR affects the underlying mechanism in rodents. Before the analysis starts, there will be an introduction about the DR regime and survival curves.

2. Introduction into dietary restriction research

2.1 Dietary restriction

The essential mechanism of DR consists of limiting the overall energy intake of an animal over a prolonged period of the post-weaning lifespan (Merry, 2002). It is also called: ‘under nutrition without malnutrition’ (Merry, 2002). The total amount of calories derived from carbohydrates, fat and proteins is reduced to 30%-60% below ad libitum intake (Merry, 2002). The extension of lifespan can approach 50% in rodents (Merry, 2002). Analysis of Merry (2002) on 14 published studies using rats and mice under varying degrees of severity of energy restriction show a significant correlation between the amount of restriction and

subsequent survival (Merry, 2002) (Fig. 1). The more an animal becomes restricted in dietary components, the longer the animal is likely to survive. A positive correlation has been found between the duration of DR and the maximum lifespan recorded (Fig. 2) The longer an animal is maintained on DR, the longer the animal is likely to survive. Taking the degree and duration of DR together: the longer the duration and the greater the degree of DR feeding during the post weaning period of lifespan, the greater the effect on survival (Merry, 2002).

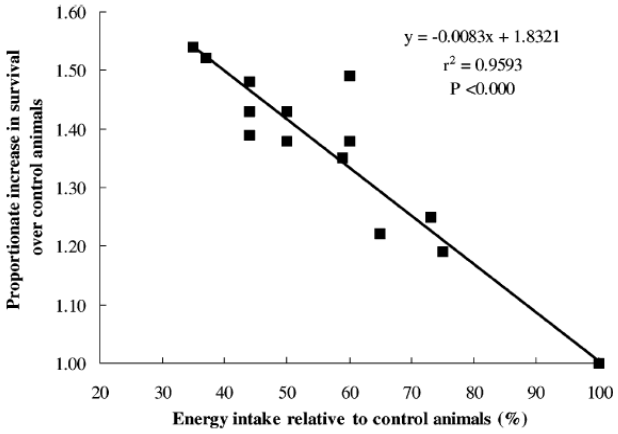

Figure 1. A negative relationship can be demonstrated between the intensity of the diet and in maximum life-span recorded. This data is taken from 24 published studies using the DR model in rodent species. An index of 1 is the survival in the non restricted animals. (From Merry,B.J., 2002, The International Journal of Biochemistry & Cell Biology, 34, 1341.)

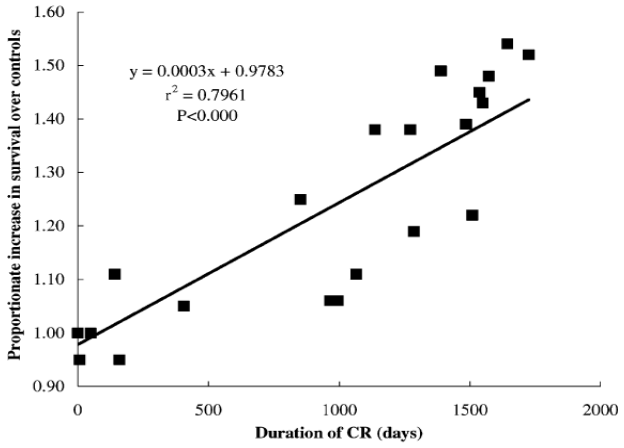

Figure 2. A positive relationship can be demonstrated between the duration of DR and the maximum life-span recorded. This data is taken from 36 published studies using DR of 40-50%. (From Merry,B.J., 2002, The International Journal of Biochemistry & Cell Biology, 34, 1342.)

Studies on the DR study a DR group, the group of interest which is restricted in a certain amount of food, and an ad libitum group, which represents the control group. Investigators that have studied the DR regime used three different approaches to study the effect of DR. One type of study changed the intensity of DR during the experimental period step by step; seeking to test the hypothesis that more restriction might yield in larger increases in longevity (Kristal & Yu, 1994). Some researchers wanted to avoid this 'stair-step' model and used a more moderate restriction that could be implemented at a constant rate throughout the course of the entire lifespan. Another type of experiments altered specific dietary components to find effects of specific dietary compounds on extension of life-span (Kristal & Yu, 1994). Although the effects of DR are attributable to the restriction of total food intake by the animal, restriction of for example protein has been found efficacious in immunological parameters (Kristal & Yu, 1994). However, this type of study is not relevant a comprehensive investigation of DR. A third set of studies implements DR in a specific period of life-span at a constant rate, mostly during adulthood. (Kristal & Yu, 1994). In this way it can be examined whether or not DR started during a specific period still beneficially can affect lifespan. This last type of studies is interesting for my thesis. If DR during adulthood still has the ability to beneficially affect lifespan than the timing of DR treatment is not of that big importance. This means DR treatment can be used in adult humans. It differs from the first approach where DR is implemented throughout the entire course of the lifespan, because that approach also takes the juvenile stage into account. Kristal & Yu (1994) argue that nutritionally retarded growth may not be beneficial and can cause problems if imposed too early. Therefore, in this

experiment I selected studies on rodents that are subjected to DR after the juvenile phase. This situation is interesting for humans, as DR can then be used as a treatment for adults.

2.2 Morality parameters

Mortality rates of DR and ad libitum groups are obtained in studies studying DR. Mortality rates are useful for efficient summarizing of data and to see the influence of a treatment on lifespan. From mortality rates it is possible to calculate mortality parameters. Mortality parameters are useful for answering the question how differences in lifespan are generated. Two important mortality parameters for studying the DR regime are the risk of death, and the rate at which mortality increases with age, also known as the rate of aging. These parameters affect other mechanisms trough which extension of lifespan can be achieved. By calculating the differences in risk of death and the rate of aging between DR and ad libitum groups, suggestions can be made regarding the effect DR has on the two mortality parameters. Figure 3 distinguishes between environmental interventions, that affect either the risk of death or the rate of aging in *Drosophila*. This figure can be used as an example to illustrate how DR affects mortality in general. A group of chronically DR animals and a group switched to the DR regime from ad libitum group can be imagined in figure 3. The chronic DR group has lower mortality due to DR. The group that will switch to DR has a higher mortality, as they ate ad libitum before the switch to DR. When DR affects the initial mortality rate, and the switch to DR occurs, the mortality trajectory of the group switching to the DR regime joins the mortality trajectory of the chronic DR group (Partridge et al, 2005). This takes place rapidly and this effect is reversible (Fig. 3a). If the group switched to the DR regime were to be switched back to ad libitum diet, mortality rates of those of permanently DR flies appear. DR in *Drosophila* therefore does not change the accumulation or irreversible damage. Instead it reduces the acute risk of death (Partridge et al, 2005). Interventions that reduce the rate of aging reduce the slope of the mortality trajectory (Fig. 3b). The mortality trajectory of the ad libitum group switched to DR starts to diverge when DR is applied. Because the slope is reduced, the switched group experiences less damage due to a lower rate of accumulation of irreversible damage. However, the mortality trajectory of the switched group will never join the mortality trajectory of the chronic DR group. Previous damage due to aging will never be erased and therefore the switched group will always show higher mortality rates compared to the chronic DR group that is DR from the start.

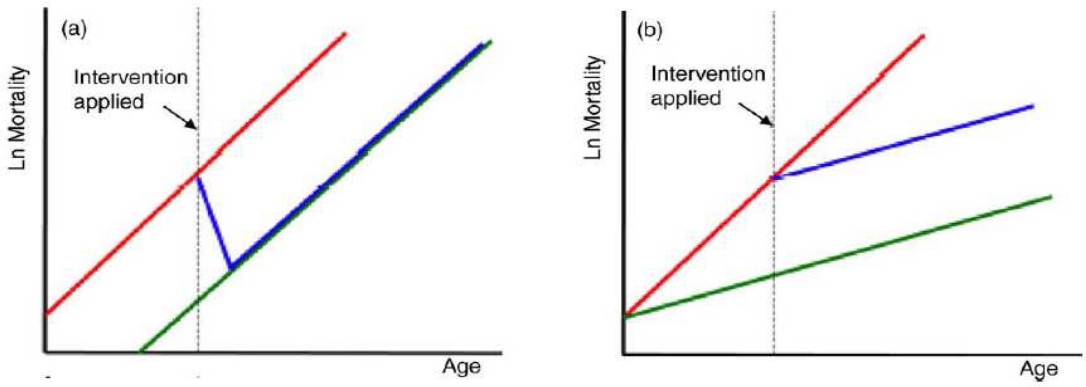

Figure 3. Schematic diagram of DR affects mortality. The green line represents a chronic DR group of animals, the red line a group on ad libitum food. The blue line represents the ad libitum group that switches to DR (a) Prediction how mortality changes if DR reduces initial mortality rate, (b) prediction how mortality changes if an DR reduces the accumulation of aging-related damage. (From Partridge, L., Pletcher, S,D., Mair, W. (2005). Dietary restriction, mortality trajectories, risk and damage. Mechanisms of aging and development 126, 35-41)

2.3 The Gompertz model

In survival analysis different functions are used to describe the mortality of a population. A number of mathematical models have been proposed to describe the relationship between age and mortality rates (Pletcher et al., 2000). I used the Gompertz model for my study, as this model also is used in earlier studies on DR. The Gompertz family of mortality models are based on the idea of a hazard that increases exponentially with age (Pletcher et al., 2000). By using the mortality rates of the DR and ad libitum group, the Gompertz model makes it possible to calculate the risk of death and rate of aging. The Gompertz model is characterized by two parameters; λ (risk of death) and γ (rate of aging). By using this model I can test how longevity is affected by DR, either by changing λ or γ, or both. The Gompertz equation is expressed as

$$m(t) = \lambda e^{\gamma t} \qquad (1)$$

where the age-dependent rate of mortality m(t), is determined by λ and γ, which affect mortality in an age-independent and age-dependent way. λ is the age independent mortality rate coefficient (i.e. the Gompertz intercept parameter). It remains constant over the lifespan and does not change the slope of the survival curve at later ages. This coefficient is estimated when development is completed and mortality from senescence is as its minimum (Pletcher et al., 2000). To illustrate the effect of λ on survival, different values for the age-independent mortality coefficient with γ held constant are shown in figure 4 (*top)*. A decrease in λ shift the curve to the right. In general the survival curves run parallel which means that the risk of death is lowered by the same proportion. As λ becomes smaller, survival becomes greater and starts to decrease sharply at the end of the lifespan.

γ is the exponential Gompertz mortality rate coefficient and is age-dependent. Attenuation of the accrual rate of oxidative stress with age is the most prominent molecular mechanism so far proposed that explains why the chance of survival decreases as age increases (Merry, 2002). γ is interpreted as a measure of intrinsic susceptibility to withstand this oxidative stress, or accumulated damage (Kristal and Yu, 1994). Therefore γ is interpreted as the rate of aging. When the Gompertz equation is plotted, γ is the slope of the survival curve. When this curve increases to approach a positive slope, then manifestation of senescence may be expected. On the other hand, when there is a negative slope the rate of senescence is positively influenced (Pletcher et al., 2000). The effect of γ is also illustrated in figure 4 (*bottom*). By changing the values for γ and

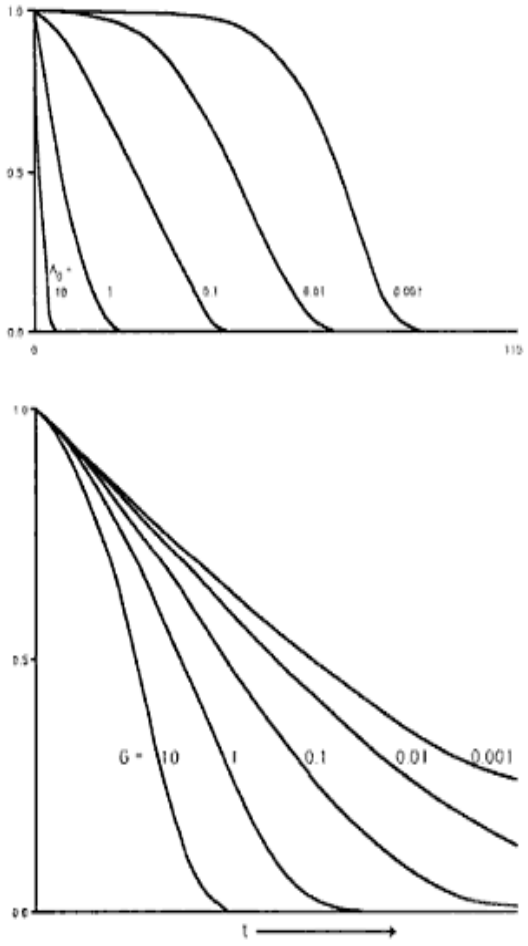

Figure 4. Survival curves according to the Gompertz model. *Top*, survivorship versus age, plotted with different values of λ with γ held constant. *Bottom*, survivorship versus age, plotted with different values of γ with λ held constant. (From Finch, C.E. 1990. Longevity, Senescence and the Genome. Chapter 1.4 : Mortality rates and life spans. 12-33.

keeping λ held constant, figure 4 shows that a decrease in γ results in greater survival. The survival curves do not run parallel, and therefore the rate of aging is not affected by the same proportion. Decreasing γ provides evidence that the rate of senescence is positively influenced (Partridge et al., 2005). As γ reflects the rate of aging, a decrease in γ due to DR results in a slower rate of aging.

Another descriptor of the aging process is the mortality rate doubling time (MRDT)(Pletcher et al., 2000). This descriptor is used later in this report. MRDT is another way to express γ. Changes in MRDT are also expected to reflect changes in rate of aging (Pletcher et al, 2000). In preference to γ, MRDT is a more natural unit for mortality rate acceleration since it does not vary inversely with lifespan like γ. It is measured in the same units of time as γ (Sacher, 1997). A MRDT of 3 means that the change of dying doubles about every 3 years.
It is expressed as

$$MRDT = \frac{ln(2)}{\gamma} \qquad (2)$$

3. Methods and materials

I obtained 17 studies studying the DR model in mice and rats. Other studies on the DR I found were not free accessible. The largest part of the 20 studies were not usable for my research question. For example, some studies studied how lifespan is affected when a specific dietary component is reduced in the DR regime. Other studies used mice strains with specific mutations or diseases. Different diet and mutations are not representative for the effect of DR has on rodents and therefore I excluded these studies from my analysis (Table 1). The majority of studies used male rodents; studies with female rodents I excluded from analyse. As mice and rats differ in their correspondence of DR on their mortality parameters, (Kristal & Yu, 1994) and have different life spans, I decided to study just one rodent species, namely mice. This resulted in 3 studies to review (Table 2). The total sample size of these 3 studies was 598 mice.

Table 1. Overview of studies which could not be used for analysis. The italic rows represents studies on mice, the remaining rows represent studies on rats. The last three columns indicate with a triangle when a study was not usable for analysis

Study	Research question	Diet	Mutation or disease	Extra
Ando et al., 2002	*Effect of DR on turnover rates of synaptic membrane lipid species*		▲	
Barger et al., 2003	*Effect of DR on lifespan extension in Ames and Snell dwarf mice, IGF-1 receptor mutants, Fat-specific insulin receptor knockout (FIRKO) and p66shc2/2 mice*		▲	
Berg and Simms, 1960	Effect of DR on the onset of diseases (chronic glomerulonephritis, periarteritis and myocardial degeneration) with different levels of food intake		▲	
Berrigan et al., 2002	*Effect of caloric restriction on delaying spontaneous tumorgenesis in p53- deficient mice*		▲	
Duffy et al., 2001	*Effect of the AIN-93 purified diet and dietary restriction on survival*	▲		

Harper et al., 2006	*Effect of caloric restriction in wild mice* (*Mus musculus*)		▲	
Hursting et al., 1994	*Effect of caloric restriction on spontaneous tumorgenesis in p53-knockout transgenic mice*		▲	
Merry et al., 2008	How does dietary lipoid acid supplementation mimic or block the effect of DR on lifespan	▲		
Pierri et al., 1990	Effect of DR on membrane micro viscosity and proliferative response in lymphocytes of females		▲	Female rats used
Ross, 1961	Effect of lifelong feeding of precise amounts of casein and sucrose on lifespan.	▲		
Tanaka et al., 2002	Effect of DR on survival in an outbred strain of rats (*Donryu*)		▲	Survival curve difficult to analyze. Squares were not distinguishable (overlapping)
Totter et al., 1984	Effect of food restriction and protracted gamma ray exposure on longevity		▲	This study did not study the DR regime, but showed a survival curve of a study of Yu et al (1982). This study was not accessible.
Umezawa et al., 1990	*Effect of DR on age-related immune dysfunction in the Senescence Accelerated Mouse (SAM)*		▲	
Yu et al., 1985	Effect of DR in adult life compared to early life DR or entire lifespan DR.	▲		Three different dietary protocols used, restricted in specific nutrients. Diet C for example is protein restriction without DR.

Table 2. Overview of reviewed studies.

Study	*Type of mice*	*Age (months)*	*DR protocol*
Weindruch et al, 1982	B10C3F1 strain	12	Ad libitum: 160 kcal per week. DR: 115 kcal/week in first months, thereafter 90 kcal/week.
Weindruch et al, 1986	B10C3F1 strain	1	Ad libitum: Non purified diet ad libitum. DR: 40, 50 or 85 kcal per week, each differing in feeding schedule.
Dhahbi et al, 2004	B10C3F1 strain	19	Ad libitum: 93 kcal per week DR: 77 kcal in first week, thereafter 52.2 kcal per week

Using computer software ImageJ I analyzed survival curves of the reviewed studies in table 2. These survival curves represent the survival data of mice that were subjected to DR or to ad libitum in the study. By analyzing the distance in pixels of the survival curves with ImageJ, I obtained data on the age of death for each mouse in the experiment. In this way I acquired all the mortality rates from the DR group and all the mortality rates from the ad libitum group. By converting mortality rates into survival rates, I remade the survival curves of the studies **(**Fig. 5, 6,7, presented as solid lines). The statistical software package R was used to fit the Gompertz model on the obtained survival data. Results from R resulted in values for λ and γ for the DR group, and λ and γ for the ad libitum group (Table 3). By entering the calculated λ and γ in the Gompertz model, I made a second survival curve for each group representing how survival should be according to the Gompertz model (Fig. 5,6,7 presented as dots).

By calculating the difference in λ and γ between DR and ad libitum it is possible to see how DR changes these parameters. By dividing these differences by the ad libitum condition, it is possible to see in what proportion λ and γ increased or decreased (Table 2, presented as fractional increase). Using this manipulation, the effect of DR on λ and γ can be discerned. To test the average fractional increase of λ and γ weighted for sample size , JMP software was used. This program calculated whether the fractional increase significantly differs from 0. Because each study has different sample sizes, each study contributes to both parameters in a different way. A study with large sample sizes contributes in another way to the parameters than a study with a small sample size. By taking the sample size into account, it is possible to correct for this. The resulting estimates for the average fractional contributions for λ and γ are presented in table 3 together with the corresponding p values. These findings, especially the significant findings, help to answer the question how differences in lifespan is achieved.
For discovering the overall survival of all mice I reviewed, I made a survival curve of all 598 mice (Fig. 8). I obtained the average estimates with JMP software for λ and γ of all DR and ad libitum groups. I used the Gompertz model to translate these parameters into a survival curve with one DR and one ad libitum group. Figure 8 has a large sample size which shows the effect of DR on survival.

4. Results

Data obtained in this study suggest λ and γ is decreased (Table 3, average fractional increase) However, the decrease in λ is found significant (p=0.001*)(Table 4). Survival curves from figure 5,6 and 7 illustrate this. All DR survival curves shift to the right compared to ad libitum groups. This is in accordance with figure 4, where a decrease in λ causes a right shift of the Gompertz curve and survival is in proportion. A decrease in γ causes a change of the slope of the survival curve, which is less significant. Figure 6 (Weindruch et al., 1986) suggest that the more a mice is restricted in diet, the more the survival curve shifts to the right. This figure in accordance with the finding of Merry (2002), showing a relationship between intensity of diet and effect on lifespan.
Secondly, mice on DR all have increased lifespan. DR mice in figure 5 (Weindruch et al., 1982) had a mean lifespan of 49 weeks, as opposed to 39 weeks for ad libitum. Mice in figure 6 (Weindruch et al., 1986) increase their lifespan as the intensity of DR increases. Ad libitum mice had a mean lifespan of 35 weeks, opposed to 54 weeks for a 40 kcal/w diet, 53 weeks for a 50 kcal/w diet and 42 weeks for a 85 kcal/w diet. This is in accordance with previous findings of Merry et al (2002), showing there is a relationship between intensity of restriction and lifespan. Figure 7 (Dhahbi et al., 2004) shows a small shift of the survival curve for DR mice. Lifespan is not extended in the DR group in contrast to the other reviewed studies.

Figure 8 summarizes the survival of all 598 mice on DR or ad libitum, weighted for sample size (dots). The significant fractional decrease of λ (table 4) is illustrated in this figure. The DR survival curve is shifted to the right compared to ad libitum. A decrease in λ causes a right shift of the survival curve according to the hypothetical Gompertz curve in figure 4. Additional survival curves are added to figure 8 (triangles) to show how much an insignificant decrease found in γ increases survival. Each triangle represents survival of a diet group with the γ value of the other diet group. The ad libitum group for example, obtained a lower value of γ of the DR group. The ad libitum group with the DR γ value is shifted to the right. However, because the fractional decrease in γ is insignificant, the shifted curve doesn't show a shallower slope. This is expected according to figure 4, where a lower γ results in a right shift of the survival curve and a shallower slope. The additional survival curve for DR also shows how much this insignificant effects adds to survival during DR.

Table 3. Summary on mortality parameters and fractional differences for mice under either DR or ad libitum feeding.

Study	*n*	*AL λ*	*DR λ*	*AL γ*	*DR γ*	*Average fractional increase λ*	*Average fractional increase γ*
Weindruch 1982 et al.	122	0,0014	0,0002	0,151	0,148	-0,562538551	-0,02003
Weindruch et al.,1986 (40kcal)	119	0,0002	1,11E-05	0,233	0,207	-0,957383356	-0,11216
Weindruch et al., 1986 (50 kcal)	120	0,0002	0,0001	0,233	0,160	-0,524885112	-0,31362
Weindruch 1986 et al.,(85 kcal)	117	0,0002	3,73E-05	0,233	0,254	-0,857374825	0,091638
Dhahbi et al., 2004	120	0,0137	0,050	0,010	0,059	-0,638623503	0,01276
Average	598	0,0005	0,0001	0,2074	0,1783	-0,6381	-0,075

Table 4. Summary of fractional estimates and p values.

	Estimate	*p value*
λ	-0.7065	0.0011*
γ	-0.0688	0.3772

Survival curves

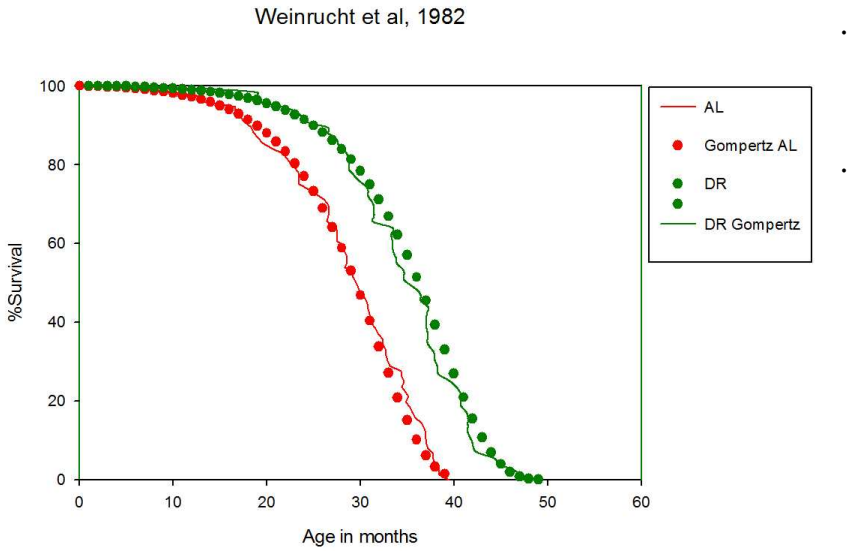

Figure 5. Survival curves of mice on a 160 kcal/w (ad libitum, AL) or 90 kcal/w(DR) diet. Each survival curve is generated from 61 mice. Circles represent the fitted Gompertz equation, the solid curve represent true results.

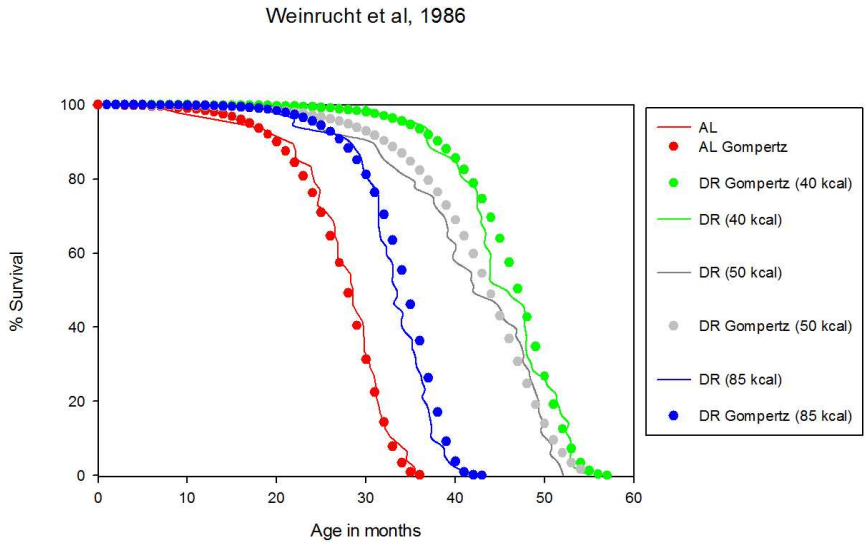

Figure 6. Survival curves of mice on a 85, 50 or 40 kcal/w (DR) diet. The ad libitum (AL) group has a unlimited amount of calories. Each survival curve is generated from approximate 60 mice. Circles represent the fitted Gompertz equation, the solid curves represent true results.

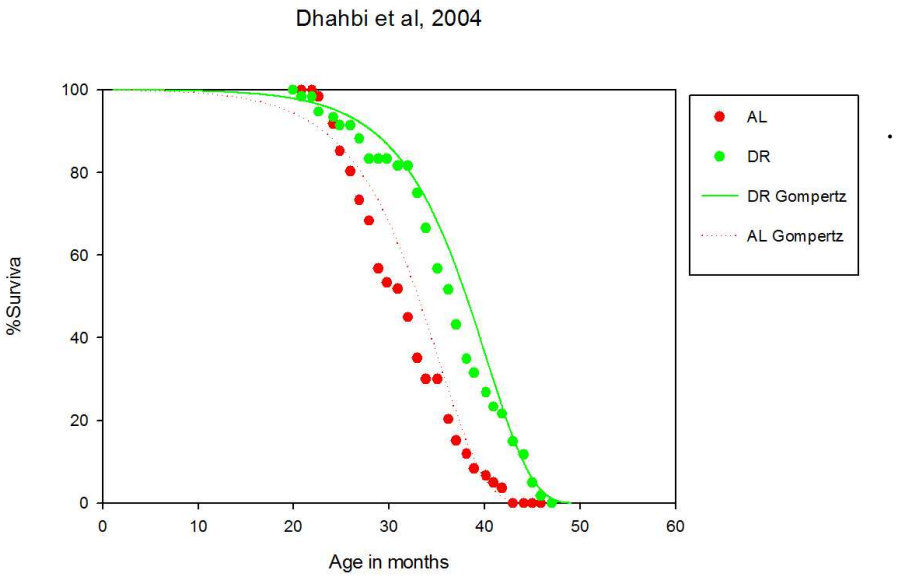

Figure 7. Survival curves of mice on a 93 kcal/w (ad libitum, AL) or 52.2 kcal/w (DR) diet. Each survival curve is generated from approximate 60 mice. Circles represent the fitted Gompertz equation, the solid curves represent true results.

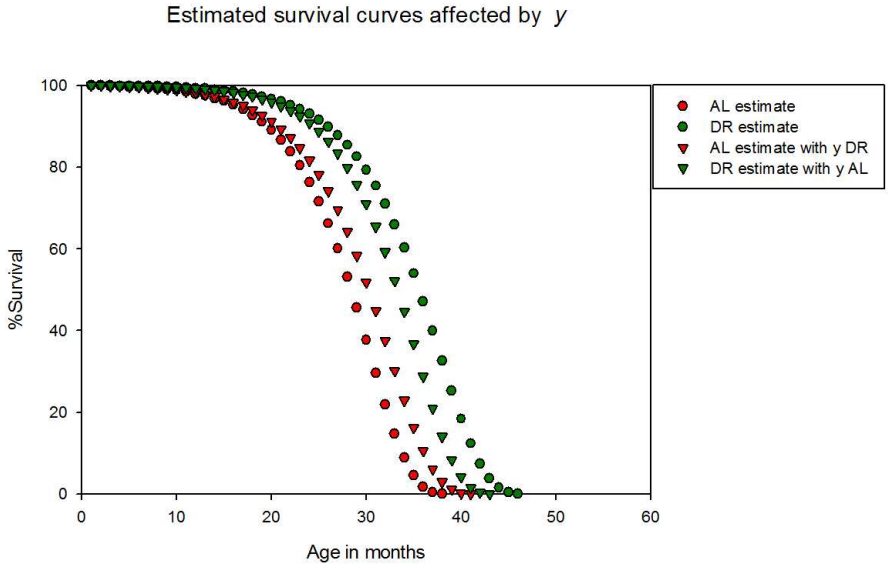

Figure 8. Estimated survival curves. The dotted curves represent the average estimates for all 598 mice on DR or AL weighted for sample size. The red triangle curve represent AL mice with the DR γ, keeping AL λ the same. The green triangle curve represent DR mice with the AL γ, keeping DR λ the same.

5. Discussion

The analytic model I used in this paper, the Gompertz model, allows to interpret differences between DR and ad libitum fed mice in terms of differences in parameters that describe mortality. With this model, I could determine whether increase in lifespan produced by DR is caused by a change in the risk of death (λ) or by a modification in the rate of increase in mortality with age (γ). This provides a unique insight into the underlying mortality changes due to DR. By using the Gompertz model I obtained λ and γ from the studies I reviewed. By studying how these parameters change during DR, I tried to determine which mechanisms affects mice during DR. Because it is the increase or decrease in mortality rates that researchers are interested in, understanding by which fraction these parameters are changed and contribute to longevity is fundamental to progress in this research.

Data examined here suggest that extension of lifespan in mice is mainly caused by a reduction in the risk of death. I found a significant decrease in the fractional increase of λ weighted for sample size (estimate= -0.7065, p=0.0011*)(Table 4). By taking the sample size into account it is possible to let all the mice contribute in the same way to the mortality parameters. The p value found for the fractional contribution of λ shows if the decrease of λ differs from 0. The null hypothesis is that DR has no effect. However, from the significant decrease in λ I conclude that DR decreases λ in mice. The decrease in λ between DR and ad libitum, compared to ad libitum is decreased by almost 71% (estimate=-0.7065*100%). The individual survival curves I reviewed illustrate this finding. All the survival curves of DR mice are shifted to the right compared to the ad libitum mice (Fig. 5,6,7). Environmental interventions that reduce the risk of death, shift the Ln-age specific mortality trajectory line to the right and results in two parallel lines (Partridge et al, 2005). The estimated survival curves obtained from all studies I reviewed also show a shift to the right and run parallel (Fig. 8). The speed of mortality in DR group is therefore in proportion to the ad libitum group. Only the time for mortality to occur is longer.

In contrast to λ, γ is not significantly affected according to my analysis. Interventions that reduce the rate of accumulation of aging related damage reduce the slope of survival curves (Partridge et al, 2005). γ, which represents the slope of the survival curve, was not significantly affected in my study(estimate= -0.0688, p=0.3772)(Table 4). This result can be illustrated by the estimated survival curves (Fig. 8). The slopes of these estimated survival curves of DR and ad libitum run almost parallel. However, DR affecting the rate of aging in mice is not excluded. Regardless, a negative intercept of -0.0688 was found, which shows a slight decrease in the rate of aging. Additional survival curves in figure 8 illustrate what affect a difference in γ would have on survival in DR and ad libitum groups. The additional survival curves are not significantly different from the estimated survival curves. Probably DR does not affect aging in a way that changes the rate of aging in a noticeable way. Data on other rodents species, like rats, suggest that DR does affect γ. Pletcher et al (2002) reanalyzed data from a previous published experiment of Yu et al (1982) on 230 rats of which 115 in the DR category and 115 in the ad libitum fed category. Difference in γ accounts for nearly all of the difference in longevity between the two groups whereas the difference in λ actually reduced the difference in longevity. An extensive analysis carried out by Finch et al (1990) on Gompertz parameters in a number of DR studies on rats, show DR is the most effective means by which to alter mortality rate doubling time (MRDT, see equation 2). MRDT is another measure for the rate of aging. This is important to point out as Finch concluded that the MRDT is a better indicator for the rate of aging of a species than the widely used maximum

lifespan (Finch, 1990). Finch analysis shows DR slows the time required for the age-specific mortality rate to double (Finch, 1990). This suggest the rate of ageing is lowered (Finch, 1990). However, Finch used some studies that I excluded from my analysis. These studies studied rodents with specific mutations or diseases. Because these rodents may not be comparable to healthy rodents, Finch results must be interpreted carefully. One of the studies on rats I did not analyse but was usable for analysis if I would have studies rats, is the study of Yu et al (1985). Finch analyzed this study on MRDT. Yu et al (1985) studied the effect of DR on three different age groups. Yu et al (1985) applied DR to rats after 6 weeks, between 6 weeks and 6 months and after 6 months. The latter group, which would have been interesting for my analysis as these rats are adult, have a MRDT of 0.32 compared to a MRDT of 0.19 for ad libitum, according to the analyse of Finch (1990). This means there is a 68% (0.32/0.19 * 100%= 68%) increase in time for the age-specific mortality rate to double. DR in rats can therefore be an effective means to alter the rate of aging. One DR mice group I reviewed shows a possible change in the rate of aging. The survival curve in figure 6 from Weindruch et al (1986) implicate that the rate of aging in the 50 kcal/w DR group is affected. This survival curve seems to have a shallower slope. Interestingly, this group showed the largest decrease in γ (-0.3136, Table 3). A decrease in γ results in a shallower slope according to figure 4. Maybe this decrease was large enough to cause an effect in the rate of aging by DR. Perhaps mice can be affected in their rate of aging after all. Is there another possible explanation for this finding? Investigation indicates that mice were restricted in protein content, decreased with advancing age. Earlier reports show that protein restriction increased life expectancy (Goodrick, 1978). As it was not my intention to review studies with specific restriction of nutrients, this finding may reveal the importance of protein in relation to the rate of aging. Perhaps the rate of aging depends on specific nutrients in stead of restriction of all nutrients.

Free radicals play role in the aging process and therefore DR could affect this mechanism (Kristal & Yu, 1994). Free radicals, which are atoms or molecules that are reactive due to an unpaired electron, proceed in a chain reaction that harm our cells and creates damage. The most important factor implicating free radicals play a role in the aging process, is the fact that under normal physiological conditions, the majority of these free radicals are generated endogenously from inspired O2 molecules as by products of the mitochondrial electron transport reaction (Kristal & Yu, 1994). Kristal & Yu (1994) refer to this as the Free Radical Theory of Aging. The consequential production of reactive oxygen species (ROS) causes oxidative damage to cells. This oxygen requirement for organisms makes it extremely difficult to eliminate the possibility that free radicals play a role in the aging process (Kristal & Yu, 1994). If aging is caused by free radicals, the prolongation of life through DR should be able to attenuate the free radical reaction (Kristal & Yu, 1994). How is a decrease in λ related to the process of aging? A model that helps to understand λ in relation to aging is the Setpoint model, suggested by Richardson and McCarter (1991) (Pletcher et al., 2000). This model postulates that rather than slowing the rate at which a process (accumulation of irreversible damage) increases with age, DR changes the level of the processes, thereby delaying the time it takes to reach a threshold at which the process cause lethality (Partridge et al., 2005). A lowered λ due to DR suggest that mice delay their time to reach a threshold at which free radicals cause lethality. Data on mice I reviewed suggest that the underlying process of aging proceeds at the same rate in DR and ad libitum fed mice, but the ability to cope with damage caused by aging is increased under DR. A lowered λ due to DR suggest that mice delay their time to reach a threshold at which free radicals cause lethality. A study of De Cabo et al (2004) suggest that DR induces higher resistance to oxidative stress-induced damage in the plasma membrane of the old rat liver, caused by an increase in antioxidant

capacity (De Cabo et al., 2004). The PM is involved in the response to oxidative stress. DR increased α-tocopherol and Coenzyme Q10 (CoQ10) which are lipophilic antioxidants involved in membrane protection against lipid peroxidation. Especially the CoQ-dependent NAD(P)H dehydrogenases were increased in the DR rat liver PM. As a consequence, the liver PM of DR old rats was more resistant to oxidative stress-induced lipid peroxidation than AL rats. DR therefore induces a higher capacity to oxidize NAD(H)P in the PM of old rat liver and as a result, a higher resistance to oxidative stress-induced damage is achieved. This study supports the Setpoint theory, as the ability to cope with damage caused by aging is increased in the old rat liver.

Current work on rhesus and squirrels monkeys will determine if DR also affect humans. The current majority of studies use species with a short lifespan. Until data on humans is available, data on these species can help us for extrapolation to humans. How can results on mice I reviewed be extrapolated to humans and how can they provide insight for interventions to implement in the life of humans? If DR would affect our rate of aging, it would be beneficial to apply DR early in life at a constant rate. Starting DR at an early age causes the most effect as this parameter depends on the length of DR. The longer we change our rate of aging, the more our rate of aging is affected. Undernourishment at a young age however causes more vulnerability to the risk of infectious diseases (Pletcher et al., 2000). As the major cause of death in children and youngsters are infectious diseases, DR at younger ages would be a problem. The study by Yu et al (1985) is in agreement with this. Yu et al (1985) concluded that DR initiated at 6 months of age is more beneficial than implementing DR after 6 weeks. Analyse on MRDT of Finch (1990) shows the effect on the rate of aging (MRDT 6 months= 0.32, MRDT 6 weeks to 6 months= 0.19, this is a 68% increase of MRDT in the 6 months group). Even more striking is the fact that DR implemented between 6 weeks and 6 months caused only a small increase in longevity (Yu et al., 1985). The MRDT of the 6 weeks to 6 months group analysed by Finch (1990) is 0.19, compared to a MRDT of 0.19 for ad libitum. According to the analyse of Finch the same values for MRDT implicate that the rate of aging is not affected. Overall this supports the idea that DR at young ages is not beneficial.
Results from my thesis suggest DR in mice influence the risk of death. Extrapolated to humans, DR doesn't necessarily have to be implemented early in life as it still extends lifespan of adult mice. It exerts an acute effect which means that if DR is implemented, it affects lifespan in an acute way. Therefore DR later in life is still beneficial. The question arises whether humans are willing to consider DR and whether it is important for them to extend their lifespan. The quality of life is of considerable importance for people. Eating less causes a permanent feeling of hunger which is experienced as unpleasant. People who tend to choose such a lifestyle usually are health conscious individuals who do so based on health reasons. Even so, when people do choose to consider applying DR, a calm lifestyle with relatively low energy requirements is advisable as it helps sustain DR. Therefore DR can be recommended for people that are retiring, as in this stage of life has less energy requirements. By eating less, retiring people could extend their life-span as the risk of death depends upon current nutritional status. As λ is age-independent, applying DR in late life could still lower their age of death due to an acutely reduced likelihood that age-related damage will give rise to lethality. DR in older humans could also be an important tool to help them survive the risk of late-life diseases. DR in rodents delays the appearance of many late-life diseases (Pletcher et al., 2000). DR causes lower cholesterol, lower blood pressure and low weight and these characteristic, when high levels, are often causal for late-life diseases. These health benefits are of great importance for elderly people that often cope with these problems.

Some remarks should be made considering studies using the DR regime. Each reviewed study uses other DR intensities and sometimes feeding regimes. This could have influenced λ and γ in different ways. Therefore results on λ and γ are less comparable to each other. Secondly, differences in mortality profiles between rats and certain strains of mice reflect the complex interaction of diet, feeding regime and phenotype. Responses in mice differ and appear to be strain dependent (Pletcher et al., 2000). Results from the B10C3F1 strain, which is the mice strain I reviewed, therefore does not have apply to other strains. More research on this topic is advisable for extrapolation to humans. One should also be mindful of the fact that inbred strains of rodent are not representative for animals in nature. Since lab strains are selected for rapid reproduction, it has been argued that these animals may have shorter life spans than wild strains (Koubova et al., 2003). According to this, DR could just correct for the defect that inbred strains have. The possibilities for further research are numerous. As DR is found to be effective in extending lifespan, more research on this topic is preferable if we want to extend our own lifespan.

6. Conclusion

The following conclusions emerge from the findings of this thesis. Extension of lifespan in mice due to DR is likely to be mediated by an acute reduction in risk of death and not the rate of aging. As the risk of death depends on the current nutritional status, DR only effects lifespan at the moment DR is applied. DR probably causes a better ability to cope with oxidative damaged caused by aging. Extrapolating these results to humans means that DR even at adulthood can be beneficial and can yield great health benefits. The best time to apply DR treatment in humans would be at a later age as this stage of life has less energy requirements and DR therefore can longer be sustained.

Acknowledgements
I want to thank M.J.P Simons, for his professional comments on my thesis and support.

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