STUDY OF DIETARY RESTRICTION RESPONSE IN MALES AND FEMALES OF LABORATORY RATS (*RATTUS NORVEGICUS*)

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The response to dietary restriction in males and females was studied 120 days using 24 laboratory rats stock Wistar. Twelve rats were fed ad libitum (6 males and 6 females) and twelve rats (6 males and 6 females) were dietary restricted (quantitative as well as time restriction was combined). Body mass gain, final body mass, food intake, relative mass of visceral organs, body-growth rate and biochemical parameters of serum were followed. Significant decrease in the daily body mass gain ($P \leq 0.05$) was found in the dietary restricted males as compared to the ad libitum fed males (the difference was 87.6 ± 12.8 g of b.m.), on the other hand, the differentiation between the groups of the females was not significant. In addition, the relative mass of liver, kidneys, heart and also the length of tibia were significantly higher in the groups of the dietary restricted males than in the dietary restricted females. However, all biochemical data of the dietary restricted rats were evaluated within current physiological standards for young rats, except of total serum cholesterol, which was slightly higher in the dietary restricted females (2.07 ± 0.39 mmol.l^{-1}). From our results it appears that the investigated dietary restriction seems to be more decent for young males than females of laboratory rats.

nutrition; dietary restriction; physiological parameters; rat; sex

INTRODUCTION

The condition under which laboratory animals are maintained can influence the results of toxicological studies. For over two decades toxicologic pathologists have noted that ad libitum-overfed sedentary laboratory rodents suffer from an early onset of degenerative disease, diet-related tumours and poor survival in chronic bioassays. Animals housed in this way are not a well-controlled subject for experimental studies (Keenan, 1996).

It has been also reported by most of literature sources Hau and Van Hoosier (2003), Nold et al. (2001), IEH (2000), McDonald (1997), Keenan et al. (1996), NRC (1995) etc. that restriction of food intake increases life span, decreases the incidence of mortality, degenerative processes, neoplasias as well as cardiac, renal and endocrine diseases. Dietary restriction seems to be an appropriate method of dietary control for the rodent bioassay when used to assess pharmaceuticals for human safety (Keenan et al., 1996, 1997; Keenan 1998). Keenan (1996) considers that reduced energy intake rather than specific nutrient restriction appears to be the main process retarding aging and improving life span.

The aim of the study was to investigate dietary restriction response in young males and females of laboratory rats. The emphasis on body mass gain and some of the physiological parameters (mass of visceral organs, length of tibia) such as information on health (biochemical data of serum) was also studied.

MATERIALS AND METHODS

Animals

Twenty four males and females of laboratory rats (*Rattus norvegicus* var. *alba*) stock Wistar were used. The rats were 21 days old at the beginning of the acclimatization (the body mass 55.0 ± 5.0 g). After that, their mean body mass was 101.6 ± 11.7 g (the body mass of the males was 105.5 ± 8.2 g, the body mass of the females was 97.3 ± 7.5 g). The animals were delivered by BioTest, Konarovice, Czech Republic.

Animal housing and feeding

The studied rats were housed in a conventional facility. The housing conditions were as follows: temperature 21.0 ± 1.0 °C, relative humidity 60 ± 10%, lighting (light 07.00–17.00 h, dark 07.00–17.00 h). The experimental animals were individually kept in plastic cages (length 42 cm, width 27 cm, height 22 cm) from Velaz, Prague, Czech Republic and the bedding was supplied by Sano GmbH, Germany. Tap water was provided ad libitum through water bottles. The diet NOE H1 from Racio, Břeclav, Czech Republic was used. The nutrition composition and energy contents of this diet is summarized in Table 1. The experimental rats were divided into two groups. Control animals (n = 12) were fed ad libitum and the experimental group was dietary restricted (n = 12). Each group contained 6 males and 6 females. The dietary

Table 1. Composition of the diet NOE H1 (Racio, Breclav)

Nutrition and energy contents	Unit	Amount
Dry matter	g/kg	898.1
Crude protein	g/kg	236.4
Crude fat	g/kg	36.6
Cellulose	g/kg	44.4
Ash	g/kg	56.5
N-free extract	g/kg	524.2
Organic matter	g/kg	841.6
Gross energy	MJ/kg	21.7

restriction was based on a combination of time and quantitative restriction. It means that the food was available from 15.00 h to 07.00 h as well as 80% amout of the diet consumed by the control (ad libitum) group was given to the experimental rats. In addition, a new value of dietary restriction was recalculated every week. This value was calculated using the amount of the food intake in the control group to see relevant food intake during growth of young rats in the experiment. The whole study took 120 days.

Experimental procedures

The experimental animals were weighed between 08.00–09.00 h every week to get the average body mass gain. The average food intake was found between 08.00–09.00 h every day as well. Weigher Kern 572, GOTL. KERN SOHN, Germany (precision 0.1 g, maximum value 650.0 g) was used to measure body mass of the rats and the amount of consumed food. At the end of the experiment the animals were anaesthetized by gas anaesthesia – carbon dioxide and the blood samples were collected from the intraorbitral venous plexus to analyse blood sugar, total protein, total serum cholesterol, serum aspartataminotransferase (S-AST), serum alaninaminotransferase (S-ALT) and serum urea (S-urea). The blood collection was carried out in the morning. Next, the rats were euthanised by carbon dioxide and necropsy was done to establish the relative mass of the visceral organs i.e. liver, kidneys and heart (calculation: g of the organ per 100 g of b.m.). Mettler AE 200, METTLER, USA weigher (precision 0.0001 g, maximum value 100.0 g) was used to find the mass of organs. Also, body-growth rate was considerable in this study, therefore tibia was removed to measure its length. Biochemical data of blood serum were analysed by using dry-chemistry analyser VetTest 8008 at the State Veterinary Institute, Prague, Czech Republic. The methods of the experiment were confirmed by Ethics Committee of the Czech University of Agriculture in Prague.

Statistical analysis

Statistical programe Statgraphic Plus version 3.1. was applied to evaluate the data. The Student's *t*-test (two-samples comparison) helped to see the difference between the groups of the ad libitum fed (AL) males and the AL females as well as the groups of the dietary restricted (DR) males and the DR females. Furthermore, the difference between the groups of the AL males and the DR males as well as between the AL females and the DR females were important in the case of the body mass gain and the final body mass. The statistical significance level was $P \leq 0.05$ (*).

Table 2. Evaluation of the followed data in the males and females of the dietary restricted and the ad libitum fed rats

Parameter		Feeding	Males	Females	Evaluation
			mean (S.D.)	mean (S.D.)	
Mass of liver	(g/100 g b.m.)	ad libitum	10.4 (1.6)	7.2 (1.1)	*
		restriction	8.1 (1.2)	5.6 (0.8)	*
Mass of kidneys	(g/100 g b.m.)	ad libitum	2.5 (0.3)	1.8 (0.1)	*
		restriction	2.1 (0.2)	1.5 (0.2)	*
Mass of heart	(g/100 g b.m.)	ad libitum	1.1 (0.1)	0.9 (0.1)	*
		restriction	1.0 (0.1)	0.8 (0.1)	*
Length of tibia	(cm)	ad libitum	4.8 (0.5)	4.2 (0.3)	*
		restriction	4.8 (0.4)	4.5 (0.6)	/
Daily body mass gain	(g)	ad libitum	2.8 (0.2)	1.4 (0.1)	*
		restriction	2.2 (0.2)	1.3 (0.2)	*
Final body mass	(g)	ad libitum	442.4 (46.3)	266.8 (15.1)	*
		restriction	355.7 (20.7)	250.2 (15.9)	*
Daily food intake	(g)	ad libitum	21.8 (5.5)	17.8 (3.6)	*
		restriction	17.0 (4.8)	13.5 (4.6)	*

* $P \leq 0.05$
/ non-significant difference

RESULTS AND DISCUSSION

Body mass gain and food intake

The results of the daily body mass gain and the daily food intake of the experimental rats are summarized in Table 2. Firstly, there was an apparent difference ($P \leq 0.05$) between the body mass gain and the daily food intake in the males (daily body mass gain 2.2 ± 0.2 g, daily foot intake 17.0 ± 4.8 g) and the females (daily body mass gain 1.3 ± 0.2 g, daily food intake 13.5 ± 4.6 g) of the DR rats. Secondly, the difference of the final body mass of the DR males and the AL males is considerably more distinct (the difference – body mass gain equals to 87.6 ± 12.8 g) as compared to the final body mass of the DR females and the AL females (14.4 ± 3.3 g). The graphical demostration of the body mass gain is displayed in Fig. 1.

A similar result was obtained by Duffy et al. (2002) and Orden et al. (1999) in their longitudinal studies. There is a possibility that the tested method does not satiate both the sex of laboratory rats in common as well as the applied dietary restriction have not met nutrition requirement for young females. Whereas there was indicated that quantitative restriction combined with the time restriction was a suitable feeding technology for young males because they do not get overweight either overfat of internal organs so easily.

Food intake is in Fig. 2, which demonstrates the average daily food intake in each week of the study in the AL males and the AL females as well as in the DR groups of the males and the females. It is necessary to notice that rats are not in the habit of eating all the amount of accessible food (Olds, Olds, 1979). Therefore, there were registered differences among the desigened dosage and the actual food intake (the difference in the DR males was 2.52% and the difference in the DR females was 5.20%).

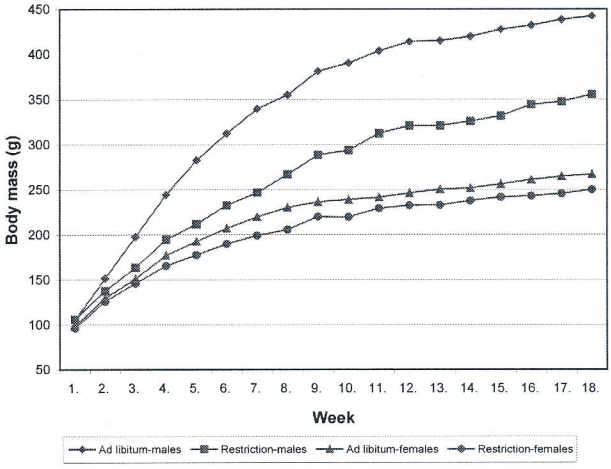

Fig. 1. The body mass gain in the dietary restricted and the ad libitum fed experimental rats, separately for males and females

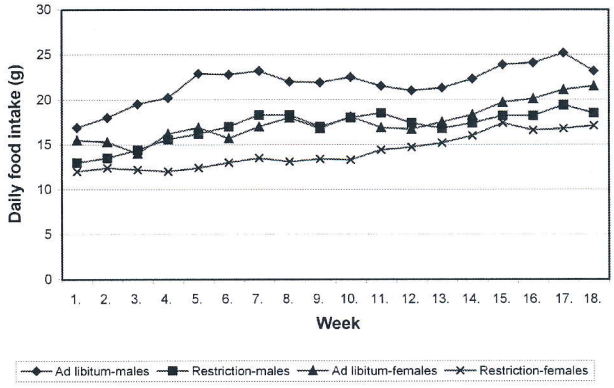

Fig. 2. The daily food intake in the dietary restricted and the ad libitum fed rats, separately for males and females in each week of the study

Mass of visceral organs and length of tibia

Table 2 gives the relative mass of the visceral organs, in particular the mass of liver, kidneys, heart and also tibial length. The different mass of the respective organs between the groups of the DR males and the DR females was found. There was detected ($P \leq 0.05$) lower mass of liver in the DR males (8.1 ± 1.2 g) as well as in the DR females (5.6 ± 0.8 g) as compared to the AL rats. It seems that lower intake of crude protein could influence neither the activity of major metabolic organ, nor its mass. The mass of organs was compared by Waynforth and Flecknell (2001), who published the mass of liver 7.2–12.0 g per b.m., kidneys 1.5–2.5 g per b.m., heart 0.8–1.3 per b.m. The results are in accordance with Kern et al. (2002), who indicated that overfeeding has impact on the incidence of liver and kidney hypertrophy in the experimental rats. The dietary restriction showed a slight but non-significant effect on tibial length between the DR males and the DR females. Furthermore, the tibial length was significantly longer in the group of the DR females (4.5 ± 0.6 cm) than in the AL females (4.2 ± 0.3 cm).

Biochemical parameters

The values of glycemia, proteinemia and serum urea, serum ALT, serum AST, total serum cholesterol are listed in Table 3. Most of the biochemical parameters did not show any important role of the dietary restriction response between the DR male and the DR female. There is one exception in the case of total serum cholesterol, which was a slightly higher ($P \leq 0.05$) in the DR females (2.07 ± 0.39 mmol.l^{-1}). It could be caused in consequence of inadequate intake of nutrients and energy, because it is known that cholesterol level is increased during starvation. This was confirmed by non-significantly higher concentration of S-urea (8.43 ± 1.31 μkat.l^{-1}) in the DR females. It is also known that higher level of S-urea can arise as a result of protein degradation when protein is used as an energy source. Most of the biochemical values in the females are slightly higher as compared to the males of the DR rats. This results in the minimum response of dietary restriction in the female

Table 3. The results of the biochemical analysis in the dietary restricted and the ad libitum fed rats

Biochemical data		Feeding	Males	Females	Evaluation
			mean (S.D.)	mean (S.D.)	
Glycemia	(mmol/l)	ad libitum	7.93 (1.13)	6.4 (1.04)	*
		restriction	7.08 (0.95)	6.27 (1.10)	/
Proteinemia	(g/l)	ad libitum	65.31 (2.50)	68.3 (2.84)	/
		restriction	64.83 (2.71)	67.67 (2.76)	/
S-Urea	(mmol/l)	ad libitum	8.88 (1.58)	7.46 (1.22)	/
		restriction	7.66 (1.53)	8.43 (1.31)	/
S-ALT	(mkat/l)	ad libitum	0.83 (0.15)	0.8 (0.17)	/
		restriction	0.73 (0.13)	0.71 (0.14)	/
S-AST	(mkat/l)	ad libitum	2.04 (0.52)	2.61 (0.51)	/
		restriction	1.84 (0.49)	1.92 (0.40)	/
S-Cholesterol	(mmol/l)	ad libitum	2.03 (0.27)	1.98 (0.55)	/
		restriction	1.77 (0.30)	2.07 (0.39)	*

* $P \leq 0.05$
/ non-significant difference

group of animals. However, the dietary restriction (on the 80% level of ad libitum feeding) was not too sufficient to alter the level of conventional biochemical parameters. Therefore, all of the obtained values were compatible with the current physiological standards for males and females of laboratory rats stock Wistar (Hau, Van Hoosier, 2003; Fox et al., 2002 and another information are provided by www.unmc.edu/Education /Animal/guide/ appenD1html.).

The animals in general appeared to be affected neither by the experimental regim, nor by other environmental factors.

CONCLUSION

Generally, this investigation provides evidence that the males and the females of the experimental rats reacted differently to the investigated feeding method. From our results it appears that the applied dietary restriction has a distinctly higher impact on the males. The tested dietary restriction prevented the young males of the rats from getting overweight and possessing a mass increase of individual organs (inclusive an increasing in organs fat). Also the analysed biochemical data were compatible with current physiological standards for laboratory rats stock Wistar. The result of this study indicated that dietary restriction could be a benefitial feeding method in rats nutrition. It might be the same situation in the case of another species of laboratory rodents, whereas this could be the work for further study.

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Studium vlivu restrikčního krmení na samce a samice laboratorního potkana (*Rattus norvegicus*).

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Je známo, že restrikce krmení pozitivně ovlivňuje délku života a snižuje výskyt některých degenerativních, rakovinných, srdečních, ledvinných a endokrinních onemocnění. V této studii jsme se zaměřili na pozorování účinku zvolené restrikce krmení na samce a samice laboratorního potkana (*Rattus norvegicus*).

Statisticky prokazatelně rozdílný denní přírůstek tělesné hmotnosti ($P \leq 0,05$) byl zaznamenán mezi samci (2,2 ± 0,2 g) a samicemi (1,3 ± 0,2 g). Dále jsme sledovali relativní hmotnost vnitřních orgánů, tj. jater, ledvin, srdce, a délku kosti holenní. Hmotnost jater u restrikčně krmených samců (8,1 ± 1,2 g) a restrikčně krmených samic (5,6 ± 0,8 g) byla nižší ve srovnání se skupinami samců (10,4 ± 1,6 g) a samic (7,2 ± 1,1 g) krmenými *ad libitum* ($P \leq 0,05$). Navíc kost holenní byla signifikantně delší u skupiny samic krmených restrikčně (4,5 ± 0,6 cm) než u samic krmených *ad libitum* (4,2 ± 0,3 cm). Pokud jde o sledované biochemické parametry krevního séra, byla nejvýrazněji ovlivněna koncentrace celkového cholesterolu, která byla nesignifikantně vyšší u restrikčně krmených samic (2,07 ± 0,39 $mmol.l^{-1}$) než u samců (1,77 ± 0,30 $mmol.l^{-1}$). Tato skutečnost může být důsledkem neadekvátního příjmu živin a energie. Tuto domněnku by potvrzoval i fakt, že S-urea byla také statisticky neprůkazně vyšší (8,43 ± 1,31 $\mu kat.l^{-1}$) u restrikčně krmených samic, neboť je známo, že v případě hladovění se jako zdroj energie využívají proteiny.

Z výsledků této studie je zřejmé, že experimentální zvířata reagovala na testovaný typ restrikce odlišně. Navíc se prokázalo, že prostřednictvím této metody krmení nedochází k překrmování zvířat a následnému výskytu nežádoucí nadváhy, a to při zachování fyziologických hodnot biochemických ukazatelů krevního séra.

výživa; restrikce krmení; fyziologické údaje; potkan; pohlaví

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