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Low Methionine Ingestion by Rats Extends Life Span

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ABSTRACT Dietary energy restriction has been a widely used means of experimentally extending mammalian life span. We report here that lifelong reduction in the concentration of a single dietary component, the essential amino acid L-methionine, from 0.86 to 0.17% of the diet results in a 30% longer life span of male Fischer 344 rats. Methionine restriction completely abolished growth, although food intake was actually greater on a body weight basis. Studies of energy consumption in early life indicated that the energy intake of 0.17% methionine–fed animals was near normal for animals of their size, although consumption per animal was below that of the much larger 0.86% methionine–fed rats. Increasing the energy intake of rats fed 0.17% methionine failed to increase their rate of growth, whereas restricting 0.85% methionine–fed rats to the food intake of 0.17% methionine–fed animals did not materially reduce growth, indicating that food restriction was not a factor in life span extension in these experiments. The biochemically well-defined pathways of methionine metabolism and utilization offer the potential for uncovering the precise mechanism(s) underlying this specific dietary restriction–related extension of life span. J. Nutr. 123: 269–274, 1993.

INDEXING KEY WORDS:

- *dietary restriction* • *life span extension*
- *aging* • *methionine* • *rats*

As first shown by McCay more than 50 years ago (McCay 1935) and by many others since, the most effective and most widely used experimental means of extending life span is by restriction of energy intake (Masoro 1988, Weindruch 1990). A wide variety of species have been studied, and in nearly every case a reduction in energy intake has been associated with an extension of life span. Although there is little debate about the beneficial effects of such restriction, which include such varied effects as delayed immune senescence (Eberly and Bruckner Kardoss 1989), retardation of cancer development (Cohen et al. 1988, Klurfeld et al. 1989), alterations in gene expression (Semsei et al. 1989), improved antioxidant protection (Laganiere and Yu 1989) and enhanced DNA repair (Srivastava and Busbee 1992), there remains considerable uncertainty about the mechanism(s) through which these varied effects are attained.

Early studies of energy restriction indicated that to be maximally effective in extending life span, restriction needed to be initiated early enough and severely enough to retard growth (Beauchene et al. 1986, McCay 1935). Nevertheless, beneficial effects have been reported when restriction was first imposed in adult rats (Weindruch and Walford 1982). The reduction in growth seen in many experiments in which restriction was initiated early in life is probably a marker of an alteration in some fundamental developmental and/or gerontologic process, and is seen as reduced growth only so long as such growth is possible. Apparently, once growth potential is exhausted, it is still possible to prolong life, but retarded growth no longer serves as an indicator.

In view of the growth-retarding nature of energy restriction in young animals, we have examined other nontoxic strategies for reducing growth in order to determine whether such approaches could also extend life span. We report here that feeding purified isocaloric diets deficient in the essential amino acid methionine eliminates growth and markedly improves survival of rats.

METHODS

These studies were reviewed and approved by the Institutional Animal Care and Use Committee of the Orentreich Foundation for the Advancement of Science, Inc.

To study survival of rats fed low methionine for extended periods of time, 60 Fischer 344 male rats obtained from Taconic Farms (Germantown, NY) at 4

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wk of age and prefed for 2 wk a nonpurified diet (Ralston Purina, St. Louis, MO) were randomly assigned to one of two groups receiving a purified diet containing either 0.86% or 0.17% L-methionine (**Table 1**). Groups of five animals were housed in a conventional animal facility in solid-bottomed cages lined with wood chips. Temperature was maintained at 22°C, and lighting was on for 12 h/d. Unless otherwise specified, all animals were given free access to food and acidified water throughout the study.

When food consumption was measured, the food ration for each cage of five rats was weighed at the initiation of the feeding interval, and again 48 h later, at which time the animals also were weighed. Food intake was measured twice per week during the first 2 mo of the experiment, and other cohorts were measured at later times of life.

Because pilot studies had indicated that rats fed 0.17% methionine consume less food than do animals fed 0.85% methionine in the diet, we examined the effects of energy intake per se in two types of experiments in which either 0.17% methionine–fed rats consumed an energy-dense diet to compensate for their reduced food intake, or 0.86% methionine–fed rodents were limited to the amount of food consumed by animals fed the low methionine ration.

In studies in which energy-dense diets were employed, 10 animals were fed for 3 mo a 0.17% L-methionine diet identical to that used throughout these studies, with the exception that the corn oil concentration of the diet was increased (at the expense of Solka-Floc) such that the energy density was raised from the normal level of 17.9 to 19.7 kJ/g. In 0.17% methionine–fed rats this level offsets the reduction in energy intake relative to those animals fed 0.86% methionine in the diet during the first 90 d of feeding.

To limit 0.86% methionine–fed rats to the intake of animals receiving 0.17% methionine, food intake was measured in 10 singly housed young rats receiving 0.17% methionine, and their average food intake was then offered to 10 singly housed rats receiving the 0.86% methionine ration. A third group of individually housed rats received free access to 0.17% methionine–containing diet. Initially, when the animals were growing rapidly, food intake was measured every 24 h; later, when growth had slowed, food intake was measured weekly, but feeding of the paired animals was always on a daily basis.

Statistical methods. Analysis of survival was conducted using Gehan's Wilcoxon test (Lee 1980), as implemented in True Epistat (Epistat Services, Richardson, TX). All other comparisons were performed using the Student's t test. Differences between groups were considered to be significant when $P \leq 0.01$.

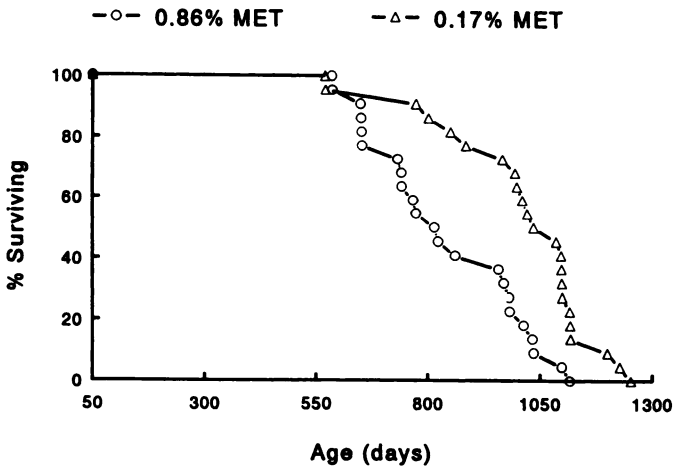

FIGURE 1 Survival of Fischer 344 male rats fed 0.86% or 0.17% methionine beginning at 42 d of age (n = 30 rats in each group).

TABLE 1

Composition of control diet[1]

Ingredient	Amount
	g/kg
L-Arginine	11.2
L-Lysine	14.4
L-Histidine	3.3
L-Leucine	11.1
L-Isoleucine	8.2
L-Valine	8.2
L-Methionine[2]	8.6
L-Threonine	8.2
L-Tryptophan	1.8
L-Phenylalanine	11.6
Glycine	23.3
Glutamic acid[2]	27.0
Dextrin	50.0
Cornstarch	436.1
Sucrose	200.0
Solka-Floc	50.0
Choline bitartrate	2.0
Vitamin mix[3]	10.0
Mineral mix[4]	35.0
Corn oil	80.0

[1]Manufactured by Ziegler Brothers (Gardners, PA) as extruded pellets, except when the energy content was raised by the addition of corn oil, in which case the diets (control and elevated energy density) were prepared as powdered meal.

[2]When the methionine content of the diet was reduced, the glutamic acid content was raised on an equal gram basis.

[3]AIN-76™ vitamin mix (AIN 1977) except that the concentration of menaquinone was 50 mg/kg.

[4]AIN-76™ mineral mix (AIN 1977).

RESULTS

Effect of dietary methionine on survival. Rats fed low methionine (0.17%) starting at 4–6 wk of age

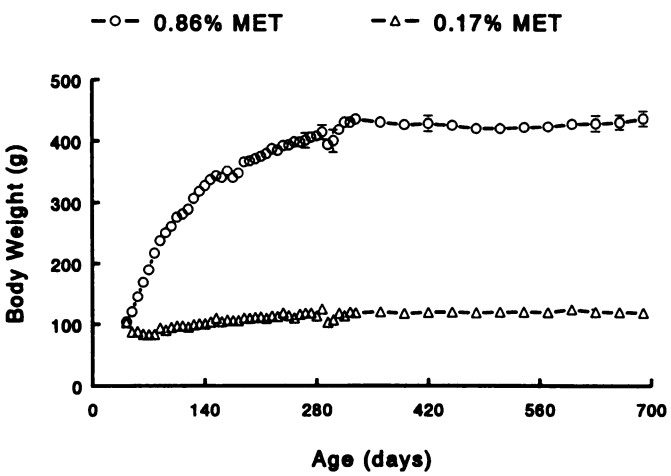

FIGURE 2 Growth of Fischer 344 male rats fed 0.86 or 0.17% methionine beginning at 42 d of age. Values are means ± SEM, n = 30 rats in each group. In some instances error bars are not visible.

showed greater median (1059 vs. 818 d) and maximum (1252 vs. 1116 d) life spans than those fed 0.86% methionine (**Fig. 1**). When the methionine concentration of the diet was reduced below 0.12% no rats survived for longer than 1 mo (data not shown).

Effect of dietary methionine on growth. Rats fed 0.86% methionine from 42 d of age gained nearly 350 g during the next 50 wk of the experiment. On the other hand, rats fed a 0.17% methionine diet from 42 d of age failed to gain weight throughout their lives (**Fig. 2**).

At the end of 90 d of feeding 0.17% methionine to rats, the reproductive organs (testes, seminal vesicles) were smaller and lung and heart were larger (relative to body size) than in rats fed 0.86% methionine. The relative sizes of the liver, prostate gland and spleen were unchanged by methionine restriction (**Table 2**).

Food intake. Food intake was measured twice weekly for the first 2 mo of feeding, again at the end of 3 mo of feeding and, in another cohort of animals, at 24 mo of age. Rats fed 0.17% methionine consumed 10% less food than control animals during the first 2 mo, 12% less food at 3 mo and 24.5% less food at 24 mo of age. Expressed in terms of body weight, however, after 3 mo of feeding, 0.17% methionine–fed animals had eaten 93% more food per gram of body weight (8.3 ± 0.4 vs. 4.3 ± 0.2 g food·d^{-1}·100 g body wt^{-1}) than rats given 0.86% methionine (**Fig. 3**). By 24 mo of age this difference was reduced to a 62% greater intake per gram of body weight in the 0.17% methionine–fed rats (data not shown).

To assess the importance of energy intake per se on growth in the 0.17% methionine–fed rats, 10 animals were fed a 0.17% methionine diet identical to that used throughout these studies, with the exception that the energy density was raised from 17.9 to 19.7

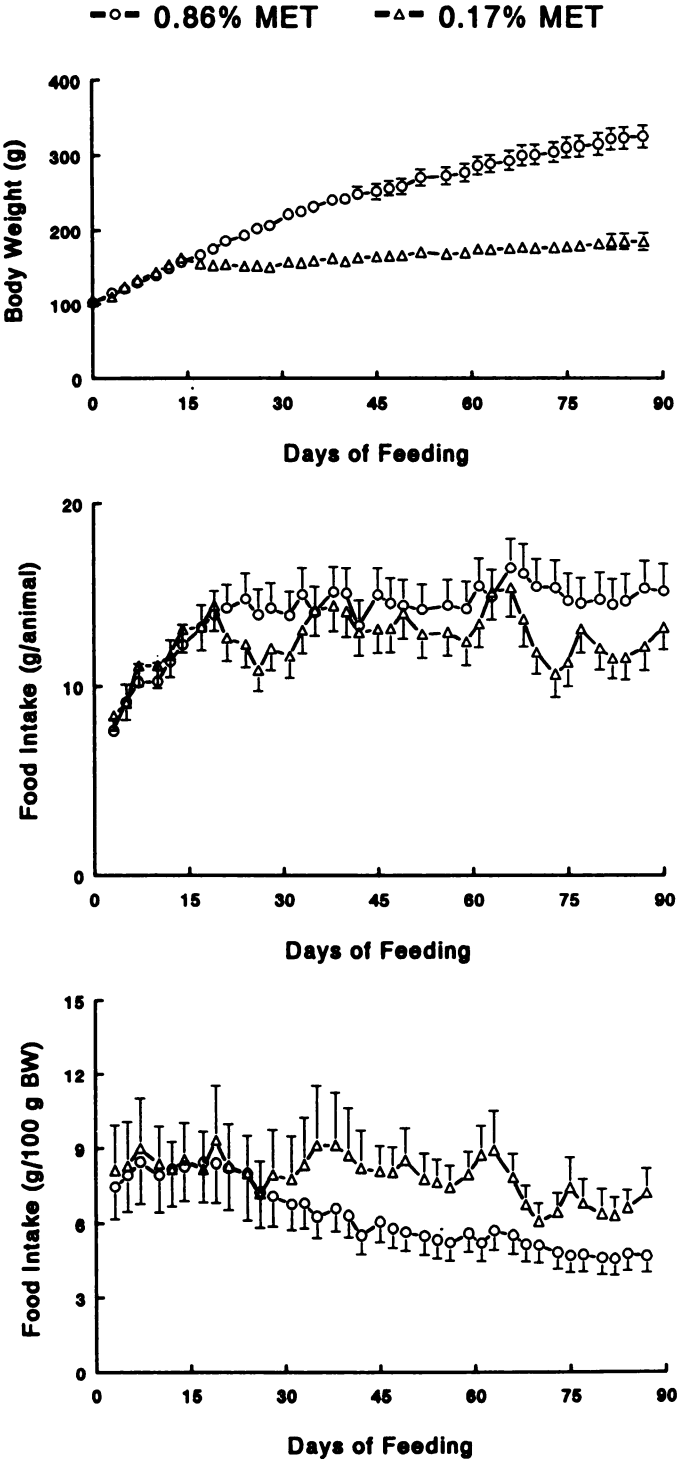

FIGURE 3 Growth and food intake of 6-wk-old male Fischer 344 rats fed 0.86% methionine for 15 d, then either 0.86 or 0.17% methionine. *Top*: Body weight. *Middle*: Food intake per animal. *Bottom*: Food intake per 100 g of body weight (BW). Values are means ± SEM, n = 10. In some instances error bars are not visible.

kJ/g. This level compensates for the lower energy intake of 0.17% methionine–fed rats at 3 mo of age. Animals consuming this energy-dense 0.17% methionine diet consumed the same amount of ration as did those fed the diet containing 17.9 kJ/g, but they

TABLE 2

Relative organ size in Fischer 344 rats consuming diets containing 0.86 or 0.17% methionine for 90 d[1]

	Diet (energy content)		
	0.86% Met (17.9 kJ/g)	0.17% Met (17.9 kJ/g)	0.17% Met (19.7 kJ/g)
Body weight, *g*	258 ± 6.0*	118 ± 6.0	123 ± 8.3
Organ weight, *g/100 g body wt*			
Testes	1.61 ± 0.04*	1.03 ± 0.02	1.16 ± 0.02
Prostate	0.06 ± 0.01	0.05 ± 0.01	0.05 ± 0.01
Seminal vesicles	0.29 ± 0.06*	0.10 ± 0.02	0.10 ± 0.01
Liver	3.56 ± 0.13	3.25 ± 0.11	3.18 ± 0.13
Spleen	0.21 ± 0.01	0.23 ± 0.01	0.22 ± 0.01
Lung	0.42 ± 0.02*	0.53 ± 0.01	0.50 ± 0.02
Heart	0.28 ± 0.01*	0.38 ± 0.12	0.50 ± 0.22
Fat pad:			
Right	1.26 ± 0.09*	0.58 ± 0.12	0.51 ± 0.10
Left	1.37 ± 0.11*	0.61 ± 0.11	0.61 ± 0.13

[1]Values are means ± SEM, $n = 10$; *$P \leq 0.01$ vs. the other two groups.

failed to gain weight during 3 mo of feeding (data not shown). Further, relative organ sizes in rats fed the energy-dense 0.17% methionine diet were not significantly different from those of animals fed the non-energy-dense 0.17% methionine diet (Table 2).

To further evaluate the role of food intake in methionine restriction, we limited 0.86% methionine–fed rats to the reduced amount of food consumed by 0.17% methionine–fed animals. Following a lag in growth, the pair-fed animals grew rapidly, and by the end of the second month of feeding they had attained the same body weight as the cohort offered free access to 0.86% methionine-containing ration; body size in these two groups was indistinguishable thereafter (**Fig. 4**).

DISCUSSION

For the past 50 years restricted energy intake has been the principal effective method for experimentally extending life span. We report here that restriction of a single dietary component, the essential amino acid methionine, also prolongs life. This observation may offer a new and valuable tool in experimental gerontology because the precise mechanism(s) underlying life span extension following energy restriction is unknown and has proven difficult to identify due to the relatively broad and ill-defined roles of energy in biological systems. On the other hand, the better-known metabolic pathways of methionine metabolism(s) offer the possibility of determining the mechanism by which this particular deprivation improves life expectancy.

In studies of the relationship between energy intake and longevity, the restricted animals are usually fed some fraction of the food consumed by unrestricted control animals. Because the restricted animals grow only to the extent that they are offered nutrient, they grow less and, consequently, over a lifetime consume less energy per animal, although the energy intake per gram of body weight is not altered. In sharp contrast, the animals in our studies were fed a palatable diet in unrestricted quantities; they cannot be called restricted in the conventional sense. Indeed, although the food intake per animal was modestly lower in those fed a 0.17% methionine diet, food intake per gram of body weight was markedly

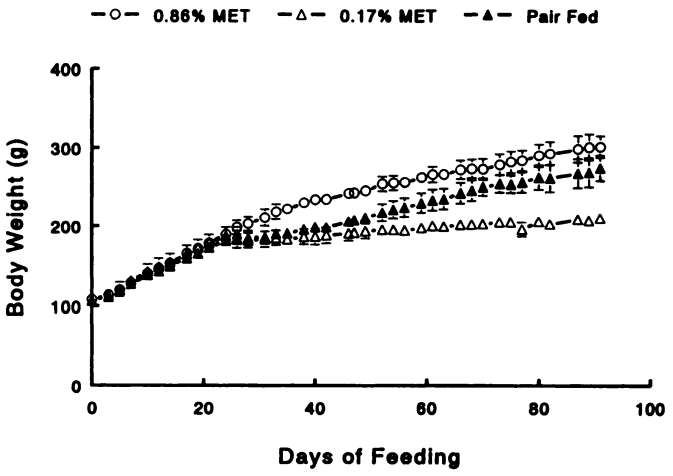

FIGURE 4 Growth of Fischer 344 male rats given free access to diet containing 0.86 or 0.17% methionine, or pair-fed 0.86% methionine–containing diet in amounts limited to those consumed by animals offered free access to diet containing 0.17% methionine. Values are means ± SEM, $n = 10$ rats in each group. In some instances error bars are not visible.

increased. Comparison of the food intake of large animals with that of smaller animals, as is conventionally done in many life extension studies, is thus flawed with respect to our studies because larger animals will obviously consume more. Rather, the determination of "restriction" should be in terms of food intake by animals of the same size. On this basis, the 0.17% methionine–fed rats in our studies consumed the same amount (or more) of energy as did rats of the same size receiving a normal level (0.86%) of methionine in their rations.

In an effort to further evaluate the role of energy intake on methionine-related life span extension, we limited 0.86% methionine–fed rats to the food intake consumed by 0.17% methionine–fed animals. The unimpaired growth of these rats argues that the degree of food intake reduction (per animal) in our long-term 0.17% methionine–fed animals is insufficient to account for the life span extension observed because, regardless of the restriction used (energy, protein or methionine), life span is extended only at nutrient intake levels that impair growth (in animals with growth potential).

In attempting to elucidate factors responsible for the life span–extending actions of dietary restriction, dietary components other than energy have been studied in the hope of identifying a single responsible nutrient or class of nutrients. However, neither a reduction in fat nor a reduction in mineral content significantly altered survival in rats when energy intake was held constant (Iwasaki et al. 1988a and 1988b). On the other hand, reduced protein intake has been associated with modest life span extension (Leto et al. 1976, Masoro et al. 1991), possibly attributable to delayed nephrotoxicity (Masoro et al. 1991). However, the magnitude of life span extension seen in rats fed 0.17% methionine in our studies was considerably greater than that attained with protein restriction. Further, with the exception of the methionine and glutamic acid (which replaces methionine in the 0.17% methionine diet) content, the amino acid composition of both the experimental and control diets was identical, and the nitrogen content was unchanged, eliminating any putative effects of the nitrogen content of the diets. We therefore do not believe that delayed nephrotoxicity explains the life span prolongation observed in rats fed a 0.17% methionine diet.

Previous reports have indicated that a diet deficient in tryptophan extends life span in rats (Ooka et al. 1988, Segall and Timiras 1976, Timiras et al. 1984). In view of our observation of life span extension when methionine is reduced in the diet, there is then a suggestion that deprivation of single essential amino acids at a level consistent with survival (but not with growth) is capable of producing life span extension. We do not yet know whether this might be a general feature of essential amino acid restriction or whether methionine acts through some mechanism unique to itself.

Thus, at this time we cannot identify the exact mechanism(s) underlying the improved survival seen in rats fed reduced levels of methionine. Indeed, many of the predicted actions of prolonged sulfhydryl amino acid deficiency would shorten life. That the mechanism of the life extension is not yet known should not deter the use of the methionine-restricted rat model in aging research, because energy restriction, the only other paradigm in wide use for modifying aging, is also unexplained. Further, animals raised on the restricted methionine protocol can be housed in groups and are given free access to food, minimizing housing and husbandry costs associated with such long-term studies.

It is entirely possible that energy and methionine restriction approaches to life span enhancement act through some common final pathway. Should that be the case, comparison of the physiologic and biochemical effects of these two models might reveal those pathways that are essential to enhance life span, while distinguishing them from those that are of minor importance. Further, by focusing on specific biochemical pathways it may be possible to identify more precisely the specific mechanism(s) responsible for life span extension under conditions of energy deprivation and/or decreased methionine intake.

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