

# Are mice calorically restricted in nature?

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## Summary

**An important question about traditional caloric restriction (CR) experiments on laboratory mice is how food intake in the laboratory compares with that of wild mice in nature. Such knowledge would allow us to distinguish between two opposing views of the anti-aging effect of CR – whether CR represents, in laboratory animals, a return to a more normal level of food intake, compared with excess food consumption typical of laboratory conditions or whether CR represents restriction below that of animals living in nature, i.e. the conditions under which house mice evolved. To address this issue, we compared energy use of three mouse genotypes: (1) laboratory-selected mouse strains (= laboratory mice), (2) house mice that were four generations or fewer removed from the wild (= wild-derived mice) and (3) mice living in nature (= wild mice). We found, after correcting for body mass, that *ad libitum* fed laboratory mice eat no more than wild mice. In fact, under demanding natural conditions, wild mice eat even more than *ad libitum* fed laboratory mice. Laboratory mice do, however, eat more than wild-derived mice housed in similar captive conditions. Therefore, laboratory mice have been selected during the course of domestication for increased food intake compared with captive wild mice, but they are not particularly gluttonous compared with wild mice in nature. We conclude that CR experiments do in fact restrict energy consumption beyond that typically experienced by mice in nature. Therefore, the retarded aging observed with CR is not due to eliminating the detrimental effects of overeating.**

**Key words:** body composition; body size; caloric restriction; energy; *Mus musculus*; wild mice.

## Introduction

Some researchers have speculated that the senescence-retarding effect of caloric restriction (CR) on laboratory rodents is in reality an artefact of overfeeding under captive conditions

(Cherkin, 1979; Hayflick, 1994). For example, Cutler (1982) asserted that CR returns 'the animal back to the aging rate it would normally have in its natural ecological niche' and does not extend lifespan beyond the 'normal genetic potential for the animal'. More generally, this argument posits that wild mice are chronically calorically restricted due to the difficulty of finding food in nature. Therefore, the typical laboratory protocol of restricting animals to 60% of their *ad libitum* (*ad lib*) food intake may more realistically replicate life in the field – the conditions under which mouse physiology has been selected. The hypothesis concludes that typical laboratory experiments, instead of comparing control with restricted animals, are in actuality comparing overfed animals with adequately fed ones, and, not surprisingly, the overfed ones develop a host of pathologies and die younger.

Overfeeding is assumed to be a result of unnatural continuous food availability, possibly exacerbated by the inadvertent selection of animals that are particularly gluttonous. Increased food consumption by mice leads to faster growth, earlier sexual maturation, larger body size and enhanced fertility in adulthood (Eisen *et al.*, 1980; Singleton *et al.*, 2001), and it is well documented that laboratory mice have been selected for enhanced reproductive rate compared with wild mice (Berry, 1969; Clark & Price, 1981; Miller *et al.*, 2000, 2002). Thus the conditions in a standard commercial mouse breeding facility will favour genetic variants that reproduce well and also eat more than other genotypes. This overfeeding response could conceivably be associated with decreased longevity and the development of numerous late-life pathologies (e.g. increased occurrence of spontaneous tumour formation), because of the diminished power of natural selection to winnow out alleles with detrimental effects that only become manifest late in reproductive life (Medawar, 1952; Rose, 1991). Overeating by laboratory mice compared with wild mice has been called the 'laboratory glutton' hypothesis of the CR effect (Austad, 2001).

There exists abundant evidence that wild mammals, including house mice, indeed eat less than they would prefer. Although it is not possible to restrict the caloric intake of animals in wild populations in a controlled manner, it is possible to supplement their food and thus potentially increase their caloric intake. Boutin (1990) summarized the results of 70 such studies in wild mammals, including five on house mice. Taken together, the studies find that supplemental feeding usually (but not always) increases the body mass of animals in the study area but always increases breeding intensity, either by lengthening the breeding season, increasing the fraction of reproducing females, or both. In all the house mouse studies, breeding intensity increased with supplemental feeding at least during some part of the calendar year. So wild mice apparently could, and would, eat more food if it were available, and this increased food availability would be beneficial at least in terms of reproduction.

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Importantly, although long-term caloric restriction provides many benefits to non-reproductive laboratory mice, these mice typically have ceased oestrous cycling (Nelson *et al.*, 1985). Because all existing wild mice populations must reproduce or become extinct, presumably wild mice are eating relatively more than restricted laboratory mice during at least part of the year. Alternatively, wild mice may respond to low calorie intake differently to laboratory mice, and therefore be capable of continued reproduction under energetic conditions in which laboratory mice are infertile.

### Caloric intake of mice

For clarity and concision, we term house mice that have lived under laboratory conditions for more than 20 generations 'laboratory mice'. Mice that have lived in the laboratory for four generations or fewer (too short a time for inadvertent selection to have substantially altered their genetic make-up in comparison with their wild ancestors) we call 'wild-derived mice', and mice living in nature we call 'wild mice'.

The central issue for the laboratory glutton hypothesis is not whether wild mice eat less than they would prefer, but whether laboratory mice eat substantially more than wild mice, such that a typical laboratory restriction regime really approximates the food consumption of mice in nature. The answer is not obvious *a priori*. Wild mice might not eat as much as they would like (Boutin, 1990, and references therein), but they may still eat as much or more than laboratory mice, because wild mice have many energetic demands, such as those associated with foraging, thermoregulation and avoiding predators, that laboratory mice do not. Wild mice must eat at least enough to meet these demands or they will suffer fitness consequences (e.g. decreased reproduction, death). Whether the amount that captive *ad lib* fed mice consume is less than, equal to or more than what is consumed by wild mice needs to be assessed empirically. Therefore, we compared, using data from the literature and from our own empirical studies, *ad lib* food intake of laboratory vs. wild-derived mice, in order to determine whether food intake of laboratory mice has been increased as a correlated response to selection for enhanced reproduction. To determine whether mice in nature eat less than mice fed *ad lib* in captivity, we also assessed energy use or intake of wild mice vs. captive mice (both laboratory mice and wild-derived mice).

### Measuring energy intake in nature

With the advent of the doubly labelled water technique to measure metabolic rate of wild animals (Speakman, 1997), we can now compare energy use by wild animals with that of captive ones. The doubly labelled water technique tracks the rate of disappearance of hydrogen and oxygen isotopes ( $^2\text{H}$  or  $^3\text{H}$  and  $^{18}\text{O}$ ) in blood samples after a known amount of doubly labelled water is injected into an animal. Oxygen in the body water rapidly equilibrates with oxygen in respiratory carbon dioxide. Thus, as oxygen leaves the body in both  $\text{CO}_2$  and water,

the radioactive oxygen signal declines. By contrast (making some reasonable assumptions), labelled hydrogen leaves the body only in water.  $\text{CO}_2$  production can therefore be easily calculated (and metabolic rate thus estimated) from the difference between the rates of disappearance of the oxygen and hydrogen isotopes (Speakman, 1997).

Assuming that animals over a short period will often be at, or near, a state of energy balance (energy expenditure equals energy intake), we can directly compare the energetics of mice in the laboratory and field. The assumption about animals in the field being at, or near, energy balance derives from the fact that small mammals have metabolic rates too high to be supported by stored fat for prolonged periods (Bronson *et al.*, 1991). Therefore, small mammals in the field need to receive about as much energy as they use most of the time. While 24 h may not be long enough for wild animals to show energy balance (Speakman *et al.*, 1994), 2–2.5 days (the duration of doubly labelled water experiments used in our analysis) may approach the time over which small rodents must show approximately balanced energy budgets.

Three studies to date have measured field metabolic rate in house mice. Geographical locations of these studies were southern California, southern Australia and a sub-Antarctic island (Nagy, 1987; Rowe-Rowe *et al.*, 1989; Mutze *et al.*, 1991). Metabolic rate measurements were typically made over a 2- to 2.5-day time interval in both sexes during both breeding and non-breeding seasons (Table 1). To test the laboratory glutton hypothesis we compared energy use of wild mice in two of these studies to food (energy) consumed by captive *ad lib* fed mice (see below for details). The sub-Antarctic island study is useful in indicating level of food intake in a particularly demanding environment (cold exposure).

### Effects of body mass and age on metabolism

One confounding issue in comparing different house mice studies is variation in body mass. Wild mice typically average substantially less than 20 g in body mass, with some mice as light as 9 g, depending on the population studied (Austad, 1996). Even 'giant' island mouse populations average less than 30 g in body mass (Berry *et al.*, 1978). By contrast, many laboratory mice grow to more than 30 g and outbred laboratory genotypes may reach peak body masses of as much as 40–50 g (Turturro *et al.*, 1999). In the studies we analysed, body mass varied from 13 to 14 g for wild mice, 14.2 to 20.2 g for wild-derived mice and 23 to 38.8 g for laboratory mice (Table 1). Note there is no overlap in these body masses.

Bioenergetics researchers have historically used mass-specific metabolic rate (dividing metabolism by total body mass or, when it is available, lean body mass) as a method for correcting for differences in body size. However, this approach has some statistical problems, because the relationship between metabolism and body mass is allometric, not isometric (Packard & Boardman, 1987, and references therein). Simply dividing by body mass (or lean mass) forces the metabolism-body size

**Table 1** Energy consumption/use by laboratory mice, wild-derived mice in captivity (four or fewer generations captive), wild mice in captivity and wild mice in nature

Genotype	Mass (g)	Daily metabolic rate		Sex	Age (months)	Temperature (°C)	Method	Reference
		$\text{kJ day}^{-1}$	$\text{kJ day}^{-1} (\text{g}^{0.568})^{-1}$					
Mice in captivity								
Wild-derived	19.5	45.9	8.5	M	3	23	Food intake <sup>d,e</sup>	J. Harper & S. N. Austad (unpubl.)
Wild-derived	20.2	44.9	8.2	F	5	23	Food intake <sup>d</sup>	D. M. Kristan & K. Hammond (unpubl.)
Wild-derived*	14.2	32.7	7.2	M	4 <sup>b</sup>	22	24-h respirometry	Ticu & Stoica (1971)
Wild-derived*	14.4	35.7	7.8	F	4 <sup>b</sup>	22	24-h respirometry	Ticu & Stoica (1971)
C3B10RF1	27 <sup>a</sup>	52.8	8.1	F	4.5	22	Food intake <sup>d,e</sup>	Weindruch <i>et al.</i> (1986)
MF1	38.8	69.5	8.7	Unk <sup>g</sup>	5 <sup>c</sup>	18	24-h respirometry	Speakman <i>et al.</i> (1991)
C57BL/6NNia	29 <sup>a</sup>	61.1	9.0	M	4	22	Food intake <sup>d,e</sup>	Turturro <i>et al.</i> (1999)
C57BL/6NNia	23 <sup>a</sup>	58.6	9.9	F	4	22	Food intake <sup>d,e</sup>	Turturro <i>et al.</i> (1999)
<b>C57BL/6J†</b>	<b>26<sup>a</sup></b>	<b>42.1</b>	<b>6.6</b>	<b>F</b>	<b>4.5</b>	<b>22</b>	<b>Food intake<sup>d,e</sup></b>	<b>Harrison <i>et al.</i> (1984)</b>
B6D2F1	28 <sup>a</sup>	63.6	9.6	M	4	22	Food intake <sup>d,e</sup>	Turturro <i>et al.</i> (1999)
B6D2F1	21 <sup>a</sup>	55.8	9.9	F	4	22	Food intake <sup>d,e</sup>	Turturro <i>et al.</i> (1999)
DBA/2JNia	26 <sup>a</sup>	63.6	10.0	M	4	22	Food intake <sup>d,e</sup>	Turturro <i>et al.</i> (1999)
DBA/2JNia	21 <sup>a</sup>	55.8	9.9	F	4	22	Food intake <sup>d,e</sup>	Turturro <i>et al.</i> (1999)
B6C3F1	28 <sup>a</sup>	75.3	11.3	M	4	22	Food intake <sup>d,e</sup>	Turturro <i>et al.</i> (1999)
B6C3F1	23 <sup>a</sup>	59.7	10.0	F	4	22	Food intake <sup>d,e</sup>	Turturro <i>et al.</i> (1999)
Swiss Webster	33 <sup>a</sup>	60.8	8.3	F	3	23	Food intake <sup>d</sup>	Kristan & Hammond (2000)
Mice in field								
Wild	13	39.8	9.3	M/F	4 <sup>b</sup>		DL water <sup>f</sup>	Nagy (1987)
Wild	14	45.3	10.1	M/F	4 <sup>b</sup>		DL water <sup>f</sup>	Mutze <i>et al.</i> (1991)
In demanding conditions								
Wild (cold)	19.3	65.1	12.2	M/F	4 <sup>b</sup>	5	DL water <sup>f</sup>	Rowe-Rowe <i>et al.</i> (1989)
Wild derived (lactation)	29.3	143.4	21.1	F	6	23	Food intake <sup>d</sup>	D. M. Kristan & K. Hammond (unpubl.)

<sup>a</sup>Mass estimated from graph.

<sup>b</sup>Age estimated based on median age of adult wild house mice; <sup>c</sup>age estimated based on mass–age relationship of outbred strains of laboratory mice.

<sup>d</sup>Digestible food intake (total food intake multiplied by digestibility); <sup>e</sup>digestible food intake estimated from published caloric content of diet.

<sup>f</sup>Doubly labelled water; <sup>g</sup>unknown.

\*Classified as separate species of *Mus* in some phylogenies.

†Anomalously low *ad lib* food intake compared with other studies of the same genotype. This is also the only published study in which CR did not extend lifespan in this genotype.

curve through the origin, and does not accurately describe metabolic differences among animals of differing size within the same species. To circumvent this problem, researchers now generally use statistical methods to correct for body mass effects [either analysis of covariance (ANCOVA) or analysis of variance (ANOVA) of residuals from the regression of metabolic rate on body mass], or express metabolic rate as energy per gram of body mass raised to an exponent where the exponent empirically describes the relationship between metabolism and body mass for the species being studied. For any of these three methods, using lean body mass may be preferable to whole body mass because fat is not very metabolically active (Martin & Fuhrman, 1955), and can be a substantial component of whole body mass for some individuals (especially for older *ad lib* fed laboratory mice).

We also tried to standardize mouse age as closely as possible, because older mice (at least in captivity) typically have lower lean-to-fat mass ratios than younger mice. In deciding which age to use we considered that wild mice are young by laboratory

standards. For laboratory mice, the median lifespan ranges from about 600 to 900 days, and 90% mortality typically occurs by about 900–1100 days. A variety of field studies have indicated that the median lifespan of wild mice is about 130 days with 90% mortality occurring by about 280 days (Berry & Jakobson, 1971; Phelan & Austad, 1989). Therefore, a random animal sampled from the field will be a youngster by laboratory standards.

## Results

Body mass was significantly greater for laboratory mice than either wild or wild-derived mice (ANOVA,  $F_{2,18} = 12.0$ ,  $P = 0.001$ ; Tukey's HSD test for multiple comparisons; Fig. 1), but did not differ statistically between wild and wild-derived mice. Similarly, absolute fat mass ( $F_{2,11} = 7.5$ ,  $P = 0.015$ ) as well as per cent body fat ( $F_{2,11} = 4.5$ ,  $P = 0.049$ ; Fig. 1; Table 2) were greater in laboratory mice compared with wild and wild-derived mice, with the latter two groups not differing statistically from one another. However, fat mass adjusted for body mass using ANCOVA

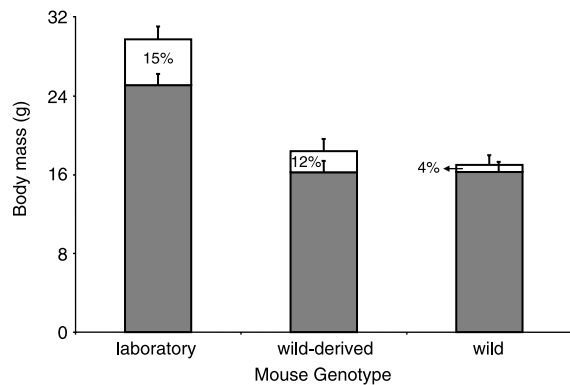
**Table 2** Body composition of laboratory mice, wild-derived mice in captivity (four or fewer generations captive born) and wild mice in nature

Genotype	Mass (g)	Dry fat mass (g)	Fat (%)	Sex	Age (months)	Temperature (°C)	Reference
<b>Mice in captivity</b>							
Wild derived	14	2.2	15	F	1.3	24	Bronson <i>et al.</i> (1991)
Wild derived	21	3.3	15	F	7.5	23	Kristan & Hammond (2003)
Wild derived	19	2.0	10	F	5	23	D. M. Kristan & K. Hammond (unpubl.)
Wild derived	17	1.6	9	F	2.5	23	D. M. Kristan (unpubl.)
Wild derived	20	1.7	9	M	2.5	23	D. M. Kristan (unpubl.)
C57BL/6J	30	6.6	22	F	1.2	22	Harrison <i>et al.</i> (1984)
Swiss Webster	34	6.0	19	F	2.5	23	Kristan & Hammond (2000a)
Swiss Webster	26	2.9	11	F	2	23	Kristan (2002b)
Swiss Webster	31	3.1	10	M	2	23	Kristan (2002b)
<b>Mice in field</b>							
Wild	19	0.7	4	M	4 <sup>a</sup>	5 <sup>b</sup>	S. N. Austad & D. M. Kristan (unpubl.)
Wild	15	0.8	5	F	4 <sup>a</sup>	5 <sup>b</sup>	S. N. Austad & D. M. Kristan (unpubl.)
<b>In demanding conditions</b>							
Wild derived (lactation)	26	0.8	3	F	6	23	D. M. Kristan & K. Hammond (unpubl.)
Swiss Webster (lactation)	38	4.3	11	F	3	23	Kristan (2002a)
Wild derived (cold)	21	2.6	12	F	3	5	Kristan & Hammond (2003)
Swiss Webster (cold)	32	6.8	21	F	2.5	5	Kristan & Hammond (2000) <sup>c</sup>

<sup>a</sup>Age estimated based on median age of adult wild house mice.

<sup>b</sup>Two male and two female mice were collected between 28 March 2002 and 17 April 2002 in Moscow, ID, when average temperature was 5 °C (range of averages: 3–6 °C).

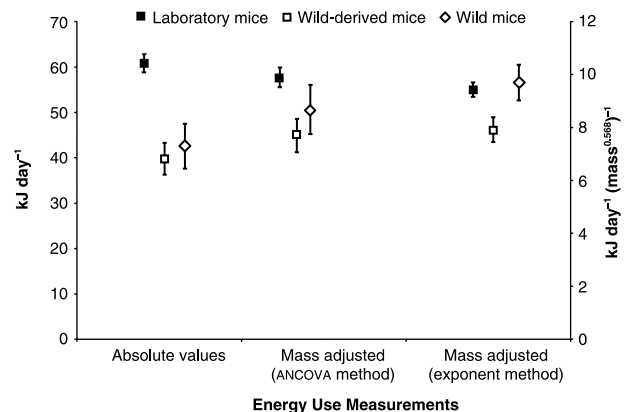
<sup>c</sup>Modified from data published in Kristan & Hammond (2000).



**Fig. 1** Body mass and composition (lean vs. fat mass) for laboratory, wild-derived and wild house mice. Per cent body fat is given for each bar. Values are means  $\pm$  1 standard error (sample sizes: laboratory,  $n = 4$ ; wild-derived,  $n = 5$ ; wild,  $n = 2$ ).

did not show significant differences among groups ( $F_{2,11} = 0.6$ ,  $P = 0.588$ ).

Because of differences in body mass, we examined both absolute energy use, and energy use standardized by body mass, either by analysis of covariance (ANCOVA) with a body mass covariate (and present least squares means  $\pm$  1 SEM), or by dividing absolute energy use by body mass raised to 0.568 (the slope of the relationship between metabolism and body mass for small mammals; Koteja, 1991). This was statistically evaluated by analysis of variance (ANOVA), followed by a Tukey HSD *post-hoc* comparison (and present arithmetic means  $\pm$  1 SEM).



**Fig. 2** Energy use for laboratory, wild-derived and wild house mice evaluated on an absolute basis and by adjusting for body mass using ANCOVA and body mass as a function of the relationship between body mass and metabolic rate. Values are means  $\pm$  1 standard error (sample sizes: laboratory,  $n = 12$ ; wild-derived,  $n = 4$ ; wild,  $n = 2$ ).

Ignoring body size, not surprisingly the much heavier laboratory mice consumed more energy than either wild-derived or wild mice ( $F_{2,18} = 16.6$ ,  $P < 0.0001$ ; Fig. 2). We found an overall significant difference between groups of mice. When we corrected for size using body mass<sup>0.568</sup>, we found a significant difference between groups ( $F_{2,18} = 4.1$ ,  $P = 0.038$ ; Fig. 2); when correcting using ANCOVA, there was a marginal statistical difference between the groups ( $F_{2,18} = 3.6$ ,  $P = 0.054$ ; Fig. 2). Using *post hoc* analyses, we found that laboratory mice consumed more energy than wild-derived mice in captivity (Tukey test of

body mass<sup>0.568</sup>:  $P = 0.04$ ; non-overlapping 95% confidence intervals for the ANCOVA), but not more than wild mice (Fig. 2). Furthermore, wild-derived mice did not consume significantly less energy than wild mice ( $P = 0.117$ ).

## Discussion

After accounting for body mass, laboratory mice do not appear to eat more than wild mice (Fig. 2). Although our sample of energy consumption from wild mice comes from only two studies, somewhat surprisingly we did find some statistically significant differences between mice in the field and the laboratory groups. These two field studies did reach remarkably similar results for field metabolic rate, however. Furthermore, one of the studies – Mutze *et al.* (1991) – was exceptionally thorough, sampling a total of 59 animals at different times of the year, during both breeding and non-breeding seasons. During some sampling periods, energy use was as high as  $12.4 \text{ kJ day}^{-1} (\text{g}^{0.568})^{-1}$  or higher than laboratory mice under any conditions measured. A third field study performed in a colder climate reported approximately the same level of energy use as the highest level found in the long-term study (Table 1). So field data from varying localities under varying conditions seem to lie within a surprisingly consistent range. Those data clearly do not support the laboratory glutton hypothesis.

The unexpectedly high values for food consumption in nature are probably due to the high energetic demands of foraging, thermoregulation, predator avoidance and reproduction. For example, wild mice measured at an average of  $5 \text{ }^\circ\text{C}$  had energy consumption that was 43% greater than the average energy consumption of laboratory mice kept at room temperature. Furthermore, wild-derived mice had 140% greater energy consumption during peak lactation than laboratory mice (Table 1; for effects of cold exposure and lactation in resting metabolism see also Hammond & Diamond, 1992; Hammond *et al.*, 1996; Kristan & Hammond, 2000).

### Inadvertent selection for increased food intake

One issue that can be addressed by the data in hand is the extent to which laboratory mice have been selected to eat more than wild-derived mice when both are fed *ad lib*. J. Harper and S. N. Austad (unpublished data) measured the *ad lib* food consumption of 3-month-old male wild-derived mice from Idaho and D. M. Kristan and K. Hammond (unpublished data) measured *ad lib* food consumption for 5-month-old female wild-derived mice from Arizona (Table 1). Our analysis shows that after accounting for body mass effects, laboratory mice consume almost 20% more energy than wild-derived mice maintained under similar captive conditions. Indeed, if we delete one study of C57BL/6J mice (Harrison *et al.*, 1984), in which food consumption for some reason was about one-third lower than in comparable studies with the same genotype, laboratory mice consume more than 30% more energy on a mass-corrected basis than wild-derived mice. Therefore, we propose that increased

food intake by laboratory mice is a correlated response to the well-documented selection for rapid growth and large litter size in laboratory mice (Berry, 1969; Clark & Price, 1981; Miller *et al.*, 2000; 2002).

### Relationship of body composition with metabolism and caloric restriction

It may be preferable to use lean body mass, rather than whole body mass, to standardize metabolism measures because body fat is not very metabolically active (Martin & Fuhrman, 1955). However, lean body mass was not available for the wild mouse studies, in which energy use was measured. It is likely that using lean body mass as a covariate in our analyses would not alter our conclusions. For example, effects of cold exposure on resting metabolism in laboratory mice were the same regardless of whether whole body mass or lean body mass was used in the analysis, despite the fact that body composition differed between cold-exposed and warm-adapted mice (Kristan & Hammond, 2000). Similarly, the effects of short-term caloric restriction on resting metabolism of laboratory mice was similar when either whole body mass or lean body mass was used as a covariate, even though the amount of body fat was less for calorically restricted than for *ad lib* fed mice (Kristan & Hammond, 2001). It should be noted that because laboratory mice are still gaining mass at 4 months of age and do not become exceptionally fat until later in life, the differences in using whole vs. lean body mass to standardize metabolism measures may become important if older laboratory mice are used.

An obvious additional consideration is whether body composition plays a role in the caloric restriction effect directly (as opposed to potential indirect effects associated with metabolism as described above). This topic has not yet been clarified. Some evidence suggests that changes in body fat are of minor importance at best in the caloric restriction effect. For example, body mass and per cent body fat have been manipulated in laboratory rats by providing them access to exercise wheels, but these treatments have far less impact on longevity than does altering caloric intake itself (Hollooszy & Schechtman, 1991; McCarter *et al.*, 1997). In addition, among calorically restricted rats, individuals with more body fat have been reported to live longer (Bertrand *et al.*, 1980). In another study suggesting that per cent body fat was not involved in the CR effect, genetically obese (*ob/ob*) mice subjected to CR exhibited 48% body fat, yet lived longer than *ad lib* fed C57BL/6J controls (22% body fat). Harrison *et al.* (1984) concluded that reduced caloric intake, not reduced body fat, was the primary component of increased longevity associated with CR. However, this conclusion may have been premature. Recent work with mice genetically engineered to lack insulin-receptor in adipose tissue (FIRKO mice) produced animals that ate as much as control animals yet were considerably leaner and lighter and lived 18% longer than controls (Blüher *et al.*, 2003). Although the authors of the FIRKO mouse paper interpreted their results as demonstrating 'the beneficial effects of reduced adiposity on the

extension of life-span . . .', clearly there would be many metabolic consequences associated with knocking out this receptor. Whether this result is attributable to reduced adiposity remains to be determined.

Additionally, Barzilai & Gupta (1999) argue that previous studies of the relationship between CR, fat mass and longevity are flawed and unable to distinguish the effects of changes in fat mass during CR on longevity. They posit that many of the systemic changes that occur with CR can be attributed to changes in circulating levels of a variety of adipocyte-derived factors (e.g. peptides, cytokines, complement factors) and that an examination of fat distribution and gene expression for factors derived from fat tissue will provide valuable information on how CR can modulate life expectancy in laboratory rodents. Therefore, although the effects of body composition may have a minor impact on basal or resting metabolism, and by extension on daily metabolic requirements, its role in whole animal response to CR remains unresolved. We are only beginning to understand the very important endocrine functions of fat tissue as they relate to physiological changes during CR. Further investigation of whether absolute fat levels are important as well as potential differences in function among different fat depots in the body that change mass differentially during CR will provide useful information towards understanding whole animal effects of CR.

### Is absolute caloric intake more important than mass-adjusted intake?

One provocative aspect of the findings presented here concerns total energy consumption per animal, i.e. energy consumption uncorrected for differences in body mass. The range of values for non-reproductive wild animals in natural populations occurring in warmer climates is approximately 40–45 kJ per animal per day whereas those for *ad lib* fed laboratory animals of approximately the same age ranged from roughly 42 to 75 kJ per animal per day (or 53–75 kJ deleting the same anomalous C57BL/6 study as previously). Reducing the *ad lib* level of laboratory consumption by 40% (a typical experimental protocol) results in restricted mice consuming between 25 and 45 kJ (or 32–45 kJ after deleting the same study) per animal per day. Therefore, ignoring body size, CR laboratory mice consume somewhat fewer, or in some cases nearly equal, calories as are expended by house mice in nature.

During long-term 40% CR, the peak body mass of restricted laboratory mice ranges from less than 40% to more than 70% smaller than *ad lib* fed animals (Turturro *et al.*, 1999). Body mass of CR laboratory mice overlaps the body masses of only the largest populations of wild mice, but is still heavier than the average mass of most wild house mice. Therefore, when body mass corrections are used, the CR laboratory mice are consuming much less than wild mice because of their relatively large body mass. Therefore, traditional CR studies are indeed limiting the restricted mice in both absolute and relative caloric intake compared with wild mice.

## Experimental procedures

We chose 4 months (about the median age of wild mice) as a reasonable age for mice in our comparisons based on the median age of wild mice in nature. In addition, whereas mice in the laboratory continue to gain weight with age, their food consumption after 4 month does not increase dramatically (Fig. 1; Turturro *et al.*, 1999). Therefore, 4 months probably also represents an age when *ad lib* food intake (our proxy for energy expenditure) per gram of body mass for laboratory mice is near its maximum.

We compared energetics data from two studies of wild house mice in nature, three studies of wild-derived or wild-caught mice and five studies that examined eight laboratory mouse genotypes (both inbred and outbred). We also compared these with several other studies of mice under extreme energy stress. Energy consumption for wild mice was measured directly with doubly labelled water. Energy consumption for captive mice was based either on 24-h energy expenditure measured by indirect calorimetry (measurement of respiratory gas exchange) or on digestible food intake (a measure of metabolizable energy). We calculated digestible food intake from total food intake multiplied by published digestibility values for each diet, converting all energy measures to metabolizable energy (kJ day<sup>-1</sup>). Where we could obtain information on diet composition (i.e. percentage fat, protein and carbohydrate), we assumed that metabolizable energy was 6% less than total calories. We determined this value based on a comparison of the relationship between total calories and metabolizable energy for seven published diets formulated by Purina Mills Inc. (average difference  $\pm$  1 standard error 6.4  $\pm$  0.9%). Because lean body mass was not available for wild mice in the published papers, we tested energy-use data using whole body mass corrections both by ANCOVA and with metabolic rate divided by body mass raised to an appropriate exponent for intraspecific studies (Koteja, 1991) analysed by ANOVA and Tukey HSD *post hoc* test for multiple comparisons.

We examined body composition for wild mice captured near Moscow, Idaho, by ether extraction of the entire carcass, and compared these values with those from four published studies of wild-derived house mice, and of three studies that examined two laboratory mouse genotypes (both inbred and outbred) (Table 2).

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