Food Restriction Differentially Affects Pituitary Hormone mRNAs throughout the Adult Life Span of Male F344 Rats1,2

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ABSTRACT Because neuroendocrine mechanisms may contribute to the antiaging effects of food restriction (FR), we measured the effect of FR on mRNAs encoding anterior pituitary (AP) tropic hormones. Slot blots or RNase protection assays were done on AP RNA from 3-, 6-, 12-, 18- and 24-mo-old male F344 rats consuming food ad libitum (AL) or food restricted (FR; to 60% of AL food intake) from 6 wk. Both AL and FR rats gained body weight during the study (P < 0.05), but FR rats weighed ~40% less (P < 0.0001). Messenger RNA levels were expressed in two ways, i.e., per total AP and per microgram total AP RNA. Proopiomelanocortin (POMC) mRNA/μg RNA was higher (P < 0.0005) in FR than in AL rats at all ages. Thyroid-stimulating hormone (TSH) β mRNA declined with age (P < 0.05) in AL but not FR rats and was reduced by FR up to 12 mo (P < 0.01). Growth hormone (GH) mRNA/μg RNA declined with age (P < 0.05) in AL but not FR rats, and total GH mRNA in the AP was reduced by FR at early ages (P < 0.05). FR reduced prolactin (PRL) mRNA and its age-related increase (P < 0.0005). Levels of luteinizing hormone (LH) β and follicle-stimulating hormone (FSH) β mRNAs did not differ between AL and FR rats until 12 mo, but thereafter rose in FR (LH β mRNA; P < 0.01, FSH β mRNA; P < 0.05). Many of these changes in gene expression corroborate previously reported hormonal changes in FR rodents and mutant mice with extended life spans, and thus provide further support for the hypothesis that an altered hormonal milieu contributes to the antiaging effects of food restriction. J. Nutr. 131: 1687–1693, 2001.

KEY WORDS: • neuroendocrine • Fischer 344 rats • food restriction

Although new genetic and pharmacologic interventions that extend mammalian life span are emerging (e.g., dw/dw mutation, deprenyl, melatonin) (1–4), food restriction (FR)5 remains the only intervention repeatedly shown to increase life span and delay a wide spectrum of age-related diseases and physiologic changes (5,6). Restriction of total energy, not reduced protein or fat energy, appears to underlie the major physiologic changes (5,6). Restriction of total energy, not reduced protein or fat energy, appears to underlie the major changes in the steady-state levels of these tropic hormones that extend mammalian life span (9). Changes in gene expression corroborate previously reported hormonal changes in FR rodents and mutant mice with extended life spans, and thus provide further support for the hypothesis that an altered hormonal milieu contributes to the antiaging effects of food restriction.

Rats that have been restricted for 6 wk exhibit altered metabolic and hormonal characteristics that persist in many cases into old age. For example, blood glucose and insulin levels are lowered by FR and remain lowered throughout the life span (10). Similarly, plasma concentrations of triiodothyronine are lowered (11) and diurnal levels of free corticosterone are elevated throughout the life span of FR rats (12). Hormonal and other FR-induced changes that are sustained throughout the life span are more likely to be factors in the antiaging action of FR than are changes that are transient, given the evidence that the antiaging effects of FR are cumulative. This study was designed to determine which changes in tropic hormone mRNAs shown to be induced by FR in young male rats were sustained throughout life. In addition, we sought to determine whether FR attenuated any age-related changes in the steady-state levels of these tropic hormone mRNAs to gain insight into the age-retarding effects of FR on the pituitary of adult male rats.

MATERIALS AND METHODS

Animals and dietary procedures. Male Fischer 344 rats were obtained at 4 wk of age from Charles River Laboratories (Kingston, NY) and housed singly in plastic cages (25.4 cm × 24.13 cm × 20.32 cm). Animals were randomized to one of two dietary groups: AL or FR. The FR group was fed to 60% of AL food intake starting at 6 wk of age and continuing to 24 mo of age. The AL group consumed food ad libitum throughout the study. Food intake was measured daily. Water was available ad libitum.

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cm) with wire-mesh floors suspended on a Hazleton-Enviro Rack System (Hazleton Systems, Aberdeen, MD) in a barrier facility (8). Rats were kept on a cycle of 12-h darkness and 12-h light (lights on at 0530 h). The presence of CAR Bacillus, murine virus antibodies (H-1 Virus, Kilham Rat Virus, Lymphocytic Choriomeningitis Virus, Parovirus, Pneumonia Virus of Mice, Rat Coronavirus/Sialodacryoadenitis Virus, Reovirus and Sendai Virus) and mycoplasma antibodies was monitored quarterly with serum samples from sentinel rats by Microbiological Associates (Rockville, MD). All tests for pathogenic organisms were negative. All procedures and experiments involving use of rats were approved by the Institutional Animal Care and Use Committee and are consistent with the NIH Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Education, the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act.

For the first 2 wk (i.e., until 6 wk of age) all rats consumed ad libitum (AL) a standard purified diet (13). At 6 wk, the ranges of their food intakes and body weights were 8.75 to 14 g and 74 to 119 g, respectively. At 6 wk, approximately half of the rats (Group AL) were randomly selected to be allowed to continue to eat this diet ad libitum until killing at 3, 6, 12, 18, and 24 mo of age. The other half (Group FR) were restricted to 60% of the mean food intake of Group AL, on a per animal basis, until killing at the same ages (Fig. 1A).

Food intake by AL rats was measured twice a week from the designated 25 rats of Group AL rats (total 150 rats for Group AL) throughout the study period and the amount ingested per day calculated. The food intake of AL rats was significantly increased with age (age main effect, P < 0.0001). FR rats were given their food allotment 1 h before the start of the dark phase of the light cycle.

Rats in both groups gained weight during the studies (P < 0.05) (Fig. 1B). The mean body weight of 3-mo-old FR rats (173 g) was 66% of that of 3-mo-old AL rats (261 g) (diet main effect, P < 0.0001). It is important to note that the FR rats used in the present study were healthy and more active than AL rats. For example, FR rats exhibit increased voluntary physical activity (14) and, compared with AL rats, live longer and exhibit delayed onset and/or reduced incidence of many age-related diseases (15,16).

Tissue collection. For measurement of proopiomelanocortin (POMC), follicle-stimulating hormone (FSH) β, luteinizing hormone (LH) β, thyroid-stimulating hormone (TSH) β, growth hormone (GH) and prolactin (PRL) RNA, tissue was collected at 0430, 0930, 1330, 1530, 1730 and 2130 h from 3-, 6-, 12-, 18- and 24-mo-old AL and FR rats. A total of 300 rats (150 AL and 150 FR rats; 5 AL and 5 FR rats at each time point for each age) were killed by decapitation. Rats were decapitated within 10 s of disturbance of their cage. Anterior pituitary dissections were dissected (the intermediate and posterior lobes were discarded), frozen in liquid nitrogen within 5 min from decapitation of the rat and stored at −70°C.

RNA preparation. RNA was extracted separately from each AP, as previously described (17). The RNA yield of each sample was determined spectrophotometrically, assuming that 1 optical density at 260 nm (OD260) unit = 40 mg/L. Samples were stored in diethylpyrocarboxylate-treated water at −70°C. The quality of the RNA extracted from each sample was monitored by 10 µL agarose formaldehyde gel electrophoresis. All samples had 260:280 ratios of ~2 and exhibited discrete 28S and 18S bands. The numbers of RNA samples used for each hormone measurement vary because the recovery of some of RNA was not sufficient to complete all measurements.

cRNA probe syntheses and Northern blot analyses. To synthesize riboprobe for POMC, a cDNA probe complementary to rat POMC mRNA from pSP64 plasmid containing a 396-bp Alul fragment from exon 3 of the rat POMC gene (18) was used. Other cDNA probes complementary to rat FSHβ, TSHβ, LHβ, GH and PRL were obtained as follows: FSHβ (rat FSH pGem 3) and TSHβ (RP21) probes were obtained from Dr. Richard A. Maurer, Oregon Health Science University (19,20). For riboprobe synthesis, an EcoRV-PstI fragment of TSHβ clone in pBR322 (RP21) was subcloned to pBluescript II ks in our laboratory. LHβ (Sp64βLHβ=1) probe was obtained from Dr. James L. Roberts, Mount Sinai School of Medicine, New York (21). GH (pRGH-1) and PRL (pSp65) probes were obtained from Dr. Beth Schachter, Mount Sinai School of Medicine, New York (22,23). For riboprobe synthesis, a PstI-KpnI fragment of GH clone in pBR322 (pRGH-1) was subcloned to pBluescript II ks in our laboratory. The riboprobes were synthesized from these cDNA probes with T7 RNA polymerase for FSHβ and TSHβ, T3 RNA polymerase for GH and SP6 RNA polymerase for POMC, LHβ and PRL following reaction conditions specified by the vendor (Promega, Madison, WI) with 32P CTP (29.6 TBq/mmol, NEN, Boston, MA). Analyses for POMC, TSHβ, FSHβ, LHβ and PRL mRNAs were carried out by slot blot. Preliminary Northern blot analyses (24) using our riboprobes revealed single discrete positive bands for these mRNAs. Because Northern blot for LHβ mRNA revealed multiple bands, LHβ message was studied by solution hybridization and electrophoresis of the hybridized sample on non-denaturing polyacrylamide gel.

Slot blot analyses for POMC, FSHβ, TSHβ, GH and PRL mRNA. Slot blots were prepared as previously described (13) with the modification of the amount of RNA used. Duplicates of each AP RNA sample (250 ng for POMC, GH and PRL, 2.5 µg for FSHβ and TSHβ) were used. To normalize values between blots for the same hormone, aliquots from pooled rat pituitary RNA (50, 100, 200 and 400 ng for POMC, GH and PRL, 0.5, 1, 2, and 4 µg for FSHβ and TSHβ) were applied in duplicate to each membrane. Liver RNA, which contains no detectable POMC, FSHβ, TSHβ, GH or PRL mRNA, was added to some of the membranes as a negative control (100 and 500 ng for POMC, 100 ng for GH and PRL, 1 µg for FSHβ and TSHβ in duplicate).

32P-labeled riboprobes for POMC FSHβ, TSHβ, GH and PRL were synthesized as described in the previous section. Hybridization was performed as previously described (24). Signal quantitation was performed with a storage phosphorimaging system (Molecular Dynamics, Sunnyvale, CA).
Solution hybridization for LHB mRNA. The solution hybridization assay was performed as described previously (25) except using 1 μg of AP RNA. To normalize values between gels, aliquots from pooled rat pituitary RNA (0.75, 1.5 and 3 μg) were hybridized with 32P-labeled probe and loaded on each gel in duplicate. Liver RNA (1 μg), which contains no detectable LHB, was also hybridized with 32P-labeled probe and loaded on a gel in duplicate as a negative control. Signal quantitation was performed with a storage phosphorimaging system.

Statistical analysis and normalization of data. The data were analyzed using two-way ANOVA (26) with two factors, age and dietary treatment. The food intake data of AL rats was analyzed by one-way ANOVA with age as a factor. The Box-Cox transformation (27) was used to meet the assumption of normality, and the Brown-Forsythe test (28) was used to test the assumption of homogeneity of variance. For the total RNA and tropic hormone mRNA analyses, a nonparametric method (Kruskal-Wallis test) (29) with adjusted P-value, P-value multiplied by the number of comparison, (30) was used to test for multiple comparisons of mean differences between dietary treatments (AL and FR) at each age (3, 6, 12, 18 and 24 mo). Because the body weight data showed normal distribution, the comparison of the body weight between dietary treatments at each age was used post-hoc test (Tukey-Kramer) (26). For each response (body weight, total RNA and mRNAs for total POMC, POMC per μg, total FSH b, FSH b per μg, total TSH β, TSH β per μg, total LH β, LH β per μg, total GH, GH per μg, total PRL and PRL per μg), the trends of means at ages were tested with nonparametric trend analysis (31) based on Kendall (32). ANOVA indicated the effect size (33) 0.20 (moderate) to 0.75 (large) in most responses; the analysis had >80% of power with P < 0.05. The only exceptions were for POMC mRNA per μg total RNA, total AP contents of POMC, TSH β and GH mRNAs, in which the effect size was < 0.20.

How to normalize data in comparisons between FR and AL rats depends on the question being asked. In this paper, mRNA levels are expressed in the following two ways: 1) total amount in the AP and 2) normalized to micrograms of total AP RNA. The former measure provides an index of the effect of FR on the total pool of substrate available to the rat for translation. The latter provides a measure of specificity of the effect of FR on a given mRNA species beyond any general effect of FR on the total RNA population, i.e., ribosomal and all mRNA species.

RESULTS

Preface. We obtained samples from rats at six time points throughout the light/dark cycle to determine whether there were diurnal variations in the expression levels of any of the mRNA species. Although several species showed a diurnal pattern (P < 0.05), in no case did FR alter that pattern (i.e., there were no significant dietary treatment by time interactions). Therefore, all data are presented as means across time points.

Total RNA. Figure 2 shows the effect of age and FR on the levels of AP total RNA, largely reflecting the ribosomal RNA pool. Total RNA levels were significantly reduced by FR at 3, 6 and 24 mo of age (Kruskal-Wallis test, P < 0.005). Total RNA increased with age (age, P = 0.0001) in both AL and FR rats, but the increase was greater in AL rats (diet, P2 = 0.0001; age × diet, P = 0.0165). Gross inspection revealed no abnormal growth in the AP of old AL rats. However, the total number of AP cells increased with age in AL rats but not in FR rats (34), and this may explain the greater increase of AP total RNA in old AL rats. These data are presented to aid interpretation of the effect of FR and age on specific mRNAs. Thus, when specific mRNAs are influenced by FR, even when normalized to total RNA abundance (e.g., POMC mRNA/μg total RNA), those changes are at least partly independent of effects of FR on the entire pool of RNA. However, when only the total AP content of an mRNA is affected by FR, the effect is likely to reflect only the effect of FR to reduce AP mass, and consequently RNA species.

POMC mRNA. Figure 3 shows the effect of age and FR on the levels of POMC mRNA/μg AP total RNA (Fig. 3A) and per AP (Fig. 3B). When expressed per microgram of total AP RNA, POMC mRNA was significantly greater in FR than AL rats throughout the life span (diet, P = 0.0001). ANOVA also showed a significant age effect (P = 0.0022), but no age × diet interaction; with advancing...
age, POMC mRNA levels increased. However, because the total amount of AP RNA was significantly greater in AL than in FR rats \((P = 0.0001)\), the AP content of POMC mRNA of AL and FR rats did not differ. Again, however, total POMC mRNA content increased significantly with age \((P = 0.0001)\), more than doubling between 3 and 24 mo in AL rats. Again, there was no interaction between age and diet for POMC mRNA per AP.

**TSHβ, GH and PRL mRNAs.** Figure 4 shows the effect of age and FR on the levels of TSHβ, GH, and PRL mRNAs in the AP. The effects of age and diet on the pattern of TSH β mRNA expression were similar whether expressed per microgram of AP RNA or total AP content. FR reduced TSH β mRNA/μg of AP RNA at 3 and 6 mo of age \((P < 0.0005)\). Total AP TSH β mRNA levels were also significantly lower in FR rats than in AL rats at 3 and 6 mo, and also at 12 mo of age \((P < 0.01)\). The reduction of TSH β mRNA by FR disappeared at later ages because of the age-related decrease of TSH β mRNA levels in AL rats. In AL rats, the levels of TSH β mRNA/μg AP RNA and total AP TSH β mRNA declined ~50% with age \((P < 0.05)\) but were stable or increased ~30% with age \((P < 0.01)\), respectively, in FR rats.

In FR rats, GH mRNA levels were stable across the life span. By contrast, in AL rats, GH mRNA/μg AP RNA decreased >50% between 3 and 24 mo \((P < 0.05)\), although the same decrease did not occur for total GH mRNA. As a consequence of its age-related decline in AL rats, GH mRNA/μg AP RNA in AL rats fell below levels in FR rats between 12 and 18 mo and remained lower thereafter \((P < 0.05)\). The AP content of GH mRNA, but not GH mRNA/μg AP RNA, was significantly lower in FR rats than in AL rats \((P = 0.0344)\). This effect of FR was restricted to the younger age groups, i.e., Kruskal-Wallis testing showed a significant decrease in total GH mRNA only at 3 mo in FR rats.

PRL mRNA increased markedly with age \((P = 0.0001)\), but FR reduced the magnitude of the age-related increase \((P < 0.0001)\); overall, it reduced PRL mRNA levels \((P = 0.0001)\). The levels of PRL mRNA/μg AP RNA were stable across the life span in FR rats, but rose progressively in AL rats from 12 mo onward compared with FR rats \((P < 0.01)\). Total AP content of PRL mRNA increased with age in both AL and FR rats \((P < 0.05)\). However, the total AP content of PRL mRNA was significantly higher in AL rats than in FR rats at all ages except 12 mo \((P < 0.05)\).

**LHβ and FSHβ mRNAs.** Figure 5 shows the effects of FR and age on the levels of the gonadotropic hormone β subunit mRNAs. The effects of age and diet on LH β and FSH β mRNAs were similar. Expressed per microgram of AP RNA, these two mRNAs were significantly elevated in FR rats compared with AL rats \((P = 0.0001)\). However, the real increases were seen only at 18 and 24 mo of age \((P < 0.05)\). Levels of both mRNAs did not differ in AL and FR rats at 3, 6 and 12 mo of age, but rose thereafter in FR rats. There were age effects on the levels of LH β mRNA/μg AP RNA \((P = 0.0338)\) and FSH β mRNA/μg AP RNA \((P = 0.0001)\). However, nonparametric trend analysis showed that these effects were restricted to FR rats \((P < 0.01\) for LH β mRNA and \(P < 0.05\) for FSH β mRNA). The AP contents of LH β and FSH β mRNAs showed patterns similar to those of the mRNAs normalized to micrograms of AP RNA, increasing in FR rats compared with AL rats \((P < 0.0001)\). However, the significant increases were apparent only at 18 and 24 mo \((P < 0.0005)\). There were age effects on the AP contents of LH β \((P = 0.0213)\) and FSH β \((P = 0.0001)\) mRNAs. This effect was due mainly to the increase with age of both mRNAs seen in FR rats \((P < 0.01\) for LH β mRNA and \(P < 0.05\) for FSH β mRNA).
AL rats, the AP contents of both mRNAs were not significantly affected by age.

**DISCUSSION**

This study is the first to demonstrate that chronic FR has marked effects on steady-state levels of all the pituitary tropic hormone mRNAs throughout the life span. These effects are hormone specific, and the effects of FR often differ at different ages. Because these pituitary mRNAs encode hormones with pleiotropic effects, including the regulation of major physiologic systems and metabolic activities, their alteration is of potential importance to the initiation and maintenance of the altered state of FR rats as well as to the antiaging mechanisms of FR.

The marked increase of the levels of total RNA in AL rats compared with FR rats after 18 mo of age was probably caused by increased mass of the AP. Pituitary weight increases with age in both AL and FR rats; however, the increase is greater in AL rats than in FR rats after 18 mo (34). Shimokawa et al. (34) reported that aging increased the total number of parenchymal cells in the anterior lobe, whereas FR decreased the total number of parenchymal cells in proportion to the pituitary weight.

POMC mRNA was the only message not down-regulated by FR at any age, whether expressed as total pituitary content or normalized to total RNA, and it was the only mRNA species elevated by FR when normalized to total RNA. POMC mRNA was also not downregulated in our previous study of 3-mo-old AL and FR rats (9). One possible explanation for persistent elevation of POMC mRNA in FR rats is its relationship to the hyperadrenocortical status of FR rats (35). Although plasma concentrations of most hormones are reduced by FR (11,36,37) at least during early adulthood, the diurnal peak of plasma corticosterone is elevated in FR rats (9). Clearly the relationship between hyperadrenocortical status and POMC mRNA and ACTH secretion in FR rats is not straightforward. There is a drive to elevate POMC mRNA levels in FR rats that is blocked distally (i.e., at the level of translation, post-translational modification or secretion), such that circulating levels of ACTH are not concomitantly elevated. It is tempting to speculate that the unique elevation of POMC mRNA may provide a substrate reserve in FR rats for greater production of ACTH and corticosterone to better handle unusual stress, which has been postulated to play a role in the natural selection for the altered status of FR rats (39).

TSH β mRNA levels were among those most profoundly affected by FR. Previously, we showed that FR significantly reduced TSH β mRNA, whether expressed as total pituitary content or normalized to total RNA, at 3 mo of age (9). TSH β mRNA levels were markedly reduced in FR rats until old age, when the age-related decline of this message in AL rats eliminated this effect. These results are consistent with reports of reduced TSH in young chronically FR rats (36) and reduced thyroid hormone levels in chronically FR rats throughout life (11,40,41). This moderately hypothyroid state may contribute to altered metabolic status of the rats; ultimately, it could play a role in its extended life span. It is noteworthy that FR shifts the cardiac myosin isozyme profiles of FR rats to those of a hypothyroid state (42,43).

As we had seen before (9), FR suppressed GH mRNA at early ages. This report reveals that this effect is not sustained as the rats age. In our previous report, the effect was significant only when the data were expressed per microgram RNA or as total amount per pituitary. In the current study, the effect was significant only when expressed as total GH mRNA content. It is noteworthy that the effect of FR on GH mRNA was much more transient than its effect on POMC and TSH mRNAs. By midlife, GH mRNA levels (per μg total AP RNA) did not differ between FR and AL rats, and by old age, levels of GH mRNA/μg RNA were higher in FR rats than in AL rats (37). Although complete age-course studies of circulating levels of GH have not been reported, our GH mRNA data roughly parallel and may thus explain in part the effect of FR on plasma GH. At early ages, FR reduces plasma GH levels (36,37,44). By contrast, in old rats, when GH levels of AL rats have fallen, FR sustains youthful pulsatility (44). It is also noteworthy that the age-related decline in GH mRNA in AL rats, which also occurred in TSH mRNA, did not occur in FR rats. Thus, FR, in addition to altering steady-state levels of hormone-encoding mRNAs in young and middle-aged rats, delays age-related changes in expression of specific pituitary mRNAs. Whether these age-retarding effects reflect actions of FR intrinsic to the pituitary or are secondary to effects of FR on aging of hypothalamic function (i.e., on hypothalamic-releasing factors) is not known. This question deserves study to map more completely the action of FR on hypothalamic-pituitary-hormone action. Identifying hypothalamic or other higher centers that are central to regulating these changes is an important step in identifying the input metabolic or other types of signals that mediate this potentially important action of FR.
FR greatly attenuated the age-related increase in PRL mRNA. In addition, as reported earlier (9), levels of PRL mRNA in even young adults were reduced by FR. The prevention of PRL mRNA elevations by FR is paralleled by the marked delay in prolactinoma development in FR rats (45,46) and may contribute to reduced mammary cancer and other deleterious consequences of elevated PRL on peripheral targets.

It is noteworthy that only the other mammalian models with life span extensions equivalent to those of FR rats are strains of dwarf mice with mutations that eliminate TSH, GH and PRL secretion (47). These are the same hormones with mRNA levels and blood levels that are reduced by FR during at least some fraction of the life span. The observation that these same hormones are also reduced by FR strengthens the notion that one or more of these hormonal changes contribute to life span extension in both mutant mice and FR rats.

Previously, we observed an effect of FR on the levels of LH β and FSH β mRNAs in 3-mo-old rats sampled at two time points during the day (9). However, in this study, involving a larger number of rats sampled at six times during the day, FR had no effect on LH β or FSH β mRNAs at 3, 6 or 12 mo of age. However, after rats reached 12 mo of age, FR not only prevented the age-related decrease in these two messages, but actually resulted in an increase in their expression levels.

Although serum gonadotropin levels have not been reported actually resulted in an increase in their expression levels.

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LITERATURE CITED

1. Armstrong, S. M. & Redman, J. R. (1991) Melatonins: a chronobiology with anti-aging properties. Med. Hypotheses 34: 300–309.
2. Carrillo, M. C., Kiltani, K., Kamai, S., Sato, Y., Miyasaka, K. & Ivy, G. O. (1994) The effect of a long-term (6 months) treatment with (-) deprenyl on antioxidant enzymes activities in selective brain regions in old female Fischer 344 rats. J. Neurochem. 60: 1333–1338.
3. Freisleben, H. J., Lehr, F. & Fuchs, J. (1994) Life span of immunosuppressed NMRI-mice is increased by deprenyl. J. Neural Transm. Suppl. 41: 231–236.
4. Miller, R. A. (1999) Kleineheer award lecture: are there genes to aging? J. Gerontol. Bi. Sci. 54A: B297–B307.
5. Masoro, E. J. (1988) Food restriction in rodents: an evaluation of its role in the study of aging. J. Gerontol. 43: B59–B64.
6. Masoro, E. J. (2000) Caloric restriction and aging: an update. Exp. Gerontol. 35: 299–305.
7. Masoro, E. J. (1992) Retardation of aging processes by food restriction: an experimental tool. Am. J. Clin. Nutr. 55 (supp. 6): 1250S–1252S.
8. Yu, B. P., Masoro, E. J. & McMahan, C. A. (1989) Nutritional influences on aging of Fischer 344 rat. 2nd ed. Physical, metabolic and longevity characteristics. J. Gerontol. 40: 657–670.
9. Han, E. S., Lu, D. H. & Nelson, J. F. (1998) Food restriction differentially affects messenger RNAs encoding the major anterior pituitary tropic hormones. J. Gerontol. Biol. Sci. 53A: B232–B329.
10. Masoro, E. J., McCarther, R., Katz, M. S. & McMahan, C. A. (1992) Dietary restriction alters characteristics of glucose fuel use. J. Gerontol. 47: B202–B208.
11. Herity, J. T., Stacy, C. & Bertrand, H. A. (1990) Long-term food restriction depresses serum thyroid hormone concentrations in the rat. Mech. Ageing Dev. 53: 9–16.
12. Sabatino, F., Masoro, E. J., McMahan, C. A. & Kuhn, R. W. (1991) Assessment of the role of the glucocorticoid system in aging processes and in the action of food restriction. J. Gerontol. Biol. Sci. 46: 817–819.
13. Han, E. S., Evans, T. R. & Nelson, J. F. (1998) Adrenocortical responsiveness to adrenocorticotropic hormone is increased in chronically food-restricted rats. J. Nutr. 128: 1415–1420.
14. McCarther, R. J., Shimokawa, I., Ikeno, Y., Higami, Y., Hubbard, G. B., Yu, B. P. & McMahan, C. A. (1997) Physical activity as a factor in the action of dietary restriction on aging: effects in Fischer 344 rats. Aging 9: 73–79.
15. Masoro, E. J., Yu, B. P., Bertrand, H. A. & Lynd, F. T. (1989) Nutritional probe of the aging process. Fed. Proc. 39: 3178–82.
16. Yu, B. P. (1994) How diet influences the aging process of the rat. Proc. Soc. Exp. Biol. Med. 205: 97–105.
17. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) Molecular Cloning—A Laboratory Manual, 2nd ed. Cold Spring Harbor Press, Cold Spring Harbor, NY.
18. Drout, J., Chamberland, M., Charron, J., Jeannotte, L. & Nemer, M. (1985) Structure of the rat proopiomelanocortin (POMC) gene. FEBS Lett. 193: 54–58.
19. Croyle, M. L. & Maurer, R. A. (1984) Thyroid hormone decreases thyrotrpin subunit mRNA level in rat anterior pituitary. DNA 3: 231–236.
20. Maurer, R. A. (1987) Molecular cloning and nucleotide sequence analysis of complementary deoxyribonucleic acid for the b-subunit of rat follicle-stimulating hormone. Mol. Endocrinol. 1: 717–723.
21. Tepper, M. A. & Roberts, J. L. (1984) Evidence for only one b-luteinizing hormone and no b-chlorionic gonadotropin gene in the rat. Endocrinology 115: 385–391.
22. Seeburg, P. H., Shine, J., Martial, J. A., Baxter, J. D. & Goodman, H. M. (1977) Nucleotide sequence and amplification in bacteria of structural gene for rat growth hormone. Nature (Lond.) 270: 486–494.
23. Croke, N. E., Cott, D., Weiner, R. L., Baxter, J. D. & Martial, J. A. (1980) Structure of cloned DNA complementary to rat prolactin messenger RNA. J. Biol. Chem. 255: 6502–6507.
24. Nelson, J. F., Bender, M. & Schachter, B. S. (1988) Age-related changes in proopiomelanocortin messenger ribonucelic acid levels in hypothalamus and pituitary of female C57BL/6J mice. Endocrinology 123: 340–344.
25. Karelus, K. & Nelson, J. F. (1992) Aging impairs estrogenic suppression of hypothalamic proopiomelanocortin messenger ribonucleic acid in the mouse. Neuroendocrinology 55: 627–633.
26. Durin, O. J. & Clark, V. A. (1987) Applied Statistics: Analysis of Variance and Regression. John Wiley & Sons, New York, NY.
27. Box, G.E.P. & Cox, D. R. (1964) An analysis of transformations. J. R. Stat. Soc. B 26: 211–252.
28. Brown, M. B. & Forsythe, A. B. (1974) Robust tests for the equality of variance. J. Am. Stat. Assoc. 69: 364–367.
29. Lehmann, E. L. (1975) Nonparametrics: Statistical Methods Based on Ranks. Holden-Day, Oakland, CA.
30. Lee, K. L., McNeer, F., Starmer, C. F., Harris, P. J. & Rosati, R. A. (1980) Clinical judgment and statistics. Lessons from a simulated randomized trial in coronary artery disease. Circulation 61: 508–15.
31. Ferguson, G. A. (1965) Nonparametric Trend Analysis. McGill University Press, Montreal, Canada.
32. Kendall, M. G. (1939) Rank Correlation Methods. Griffin, London, UK.
33. Cohen, J. (1977) Statistical Power Analysis for the Behavioral Sciences, rev. ed. Academic Press, New York, NY.
34. Shimokawa, I., Yu, B. P., Higami, Y. & Ikeda, T. (1996) Morphometric analysis of somatotropes: effects of age and dietary restriction. Neurobiol. Aging 17: 79–86.
35. Han, E. S., Levin, N., Bengani, N., Roberts, J. L., Suh, Y., Karelus, K. & Nelson, J. F. (1995) Hyperadrenocorticism and food restriction-induced life extension in the rat: evidence for divergent regulation of pituitary proopio-

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nocortin RNA and adrenocorticotropic hormone biosynthesis. J. Gerontol. 50A: B288–B294.
36. Armario, A., Montero, J. L. & Jolin, T. (1987) Chronic food restriction and the circadian rhythms of pituitary-adrenal hormones, growth hormone and thyroid-stimulating hormone. Ann. Nutr. Metab. 31: 81–87.
37. Bronson, F. H. & Heideman, P. D. (1990) Short-term hormonal responses to food intake in peripubertal female rats. Am. J. Physiol. 259: R25–R31.
38. Klebanov, S., Diais, S., Stavinoha, W., Suh, Y. & Nelson, J. F. (1995) Hyperadrenocorticism, attenuated inflammation, and the life-prolonging action of food restriction in mice. J. Gerontol. Biol. Sci. 50: B79–B82.
39. Masoro, E. J. & Austad, S. N. (1996) The evolution of the antiaging action of dietary restriction: a hypothesis. J. Gerontol. 51: B387–B391.
40. Dillman, W., Berry, S. & Alexander, N. (1983) A physiological dose of triiodothyronine normalizes cardiac myosin adenosine triphosphatase activity and changes myosin isoenzyme distribution in semistarved rats. Endocrinology 112: 2081–2087.
41. Merry, B. J. & Holehan, A. M. (1984) The endocrine response to dietary restriction in the rat. In: The Molecular Biology of Ageing (Woodhead, A. D., Blackett, A. D. & Holander, A., eds.), pp. 117–141. Plenum Press, New York, NY.
42. Klebanov, S. & Herlihy, J. T. (1987) Effect of life-long food restriction on cardiac myosin composition. J. Gerontol. A Biol. Sci. Med. Sci. 52: B184–B189.
43. Morris, G. S., Surdyka, D. G., Haddad, F. & Baldwin, K. M. (1990) Apparent influence of metabolism on cardiac isomyosin profile of food-restricted rats. Am. J. Physiol. 258: R346–R351.
44. Sonntag, W. E., Xu, X., Ingram, R. L. & D’Costa, A. (1995) Moderate caloric restriction alters the subcellular distribution of somatostatin mRNA and increases growth hormone pulse amplitude in aged animals. Neuroendocrinology 61: 601–608.
45. Fernandes, G., Chandrasekar, B., Troyer, D. A., Venkatraman, J. T. & Good, R. A. (1995) Dietary lipids and calorie restriction affect mammary tumor incidence and gene expression in mouse mammary tumor virus/v-Ha-ras transgenic mice. Proc. Natl. Acad. Sci. U.S.A. 92: 6494–6498.
46. Sylvester, P. W., Aylsworth, C. F. & Mettes, J. (1991) Relationship of hormones to inhibition of mammary tumor development by underfeeding during the “critical period” after carcinogen administration. Cancer Res. 41: 1384–1388.
47. Bartke, A. (2000) Delayed aging in ames dwarf mice: relationship to endocrine function and body size. In: The Molecular Genetics of Aging (Hekimi, S., ed.), pp. 181–202. Springer-Verlag, Berlin, Germany.
48. Amador, A., Steger, R. W., Bartke, A., Johns, A., Siler-Khodr, T. M., Parker, C. R., Jr. & Shepherd, A. M. (1985) Testicular LH receptors during aging in Fischer 344 rats. J. Androl. 6: 61–64.
49. Gruenewald, D. A., Hess, D. L., Wilkinson, C. W. & Matsumoto, A. M. (1992) Excessive testicular progesterone secretion in aged male Fischer 344 rats: a potential cause of age-related gonadotropin suppression and confounding variable in aging studies. J. Gerontol. 47: B164–B170.
50. Stokkan, K. A., Reiter, R. J., Vaughan, M. K., Nonaka, K. O. & Lerchl, A. (1991) Endocrine and metabolic effects of life-long food restriction in rats. Acta Endocrinol. 125: 93–100.
51. Bartke, A., Sweeney, C. A., Johnson, L., Castracane, V. D. & Doherty, P. C. (1985) Hyperprolactinemia inhibits development of Leydig cell tumors in aging Fischer rats. Exp. Aging Res. 11: 123–128.
52. Maeda, H., Gleiser, C. A., Masoro, E. J., Murata, I., McMahan, C. A. & Yu, B. P. (1985) Nutritional influences on aging of Fischer 344 rats: II. Pathology. J. Gerontol. 40: 671–688.