Food Restriction Differentially Affects Pituitary Hormone mRNAs throughout the Adult Life Span of Male F344 Rats

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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 mRNAs 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 rise 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.


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) 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 physiologic changes (5,6).


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4 Abbreviations used: ACTH, adrenocorticotropic hormone; AL, consumed food ad libitum; AP, anterior pituitary; FR, food restriction; FSH, follicle-stimulating hormone; GH, growth hormone; LH, luteinizing hormone; POMC, proopiomelanocortin; PRL, prolactin; TSH, thyroid-stimulating hormone.

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).
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Food intakes and body weights of rats that consumed food ad libitum (AL) or were restricted to 60% of ad libitum intake (FR). (A) Effect of age on food intake (AL; \( n = 25 \) for 3, 6, 12 mo, \( n = 24 \) for 18 mo, and \( n = 12 \) for 24 mo). AL values are means \( \pm \) SEM. FR values are 60% of AL values at each age. (B) Effect of age and food restriction on body weight (\( n = 30 \) except \( n = 31 \) for 18 mo AL, and \( n = 29 \) for 24 mo AL). Values are means \( \pm \) SEM. *Significantly different from AL (Tukey-Kramer test, \( P < 0.0001 \)).
Solution hybridization for LHB mRNA. The solution hybridization assay was performed as described previously (23) 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 β, FSH β 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 FR 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, since 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 TSHβ mRNA expression were similar whether expressed per microgram of AP RNA or total AP content. FR reduced TSHβ mRNA/μg of AP RNA at 3 and 6 mo of age (Kruskal-Wallis test, $P < 0.0005$). Total AP TSHβ 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 (Kruskal-Wallis test, $P < 0.01$). The reduction of TSHβ mRNA by FR disappeared at later ages because of the age-related decrease of TSHβ mRNA levels in AL rats. In AL rats, the levels of TSHβ mRNA/μg AP RNA and total AP TSHβ mRNA declined ~50% with age (nonparametric trend analysis, $P < 0.05$) but were stable or increased ~30% with age (nonparametric trend analysis, $P < 0.01$), 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 (nonparametric trend analysis, $P < 0.05$), 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 (Kruskal-Wallis test, $P < 0.05$ at 18 and 24 mo). The AP content of GH mRNA, but not GH mRNA/μg AP RNA, was significantly lower in FR rats than in AL rats (diet, $P = 0.0344$). 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 (age × diet, $P = 0.0001$); overall, it reduced PRL mRNA levels (diet, $P = 0.0001$). 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 (nonparametric trend analysis, $P < 0.01$ and Kruskal-Wallis test, $P < 0.01$ at 18 and 24 mo). Total AP content of PRL mRNA increased with age in both AL and FR rats (nonparametric trend analysis, $P < 0.05$). However, the total AP content of PRL mRNA was significantly higher in AL rats than in FR rats at all ages except 12 mo (Kruskal-Wallis test, $P < 0.05$).

**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 LHβ and FSHβ mRNAs were similar. Expressed per microgram of AP RNA, these two mRNAs were significantly elevated in FR rats compared with AL rats (diet effect on both mRNAs, $P = 0.0001$). However, the real increases were seen only at 18 and 24 mo of ages (Kruskal-Wallis test, LHβ mRNA; $P < 0.05$ and FSHβ mRNA; $P < 0.0005$). 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 LHβ mRNA/μg AP RNA ($P = 0.0338$) and FSHβ mRNA/μg of AP RNA ($P = 0.0001$). However, nonparametric trend analysis showed that these effects were restricted to FR rats (FR < 0.01 for LHβ mRNA and $P < 0.05$ for FSHβ mRNA).

The AP contents of LHβ and FSHβ mRNAs showed patterns similar to those of the mRNAs normalized to micrograms of AP RNA, increasing in FR rats compared with AL rats (diet effect on both, $P = 0.0001$). However, the significant increases were apparent only at 18 and 24 mo (Kruskal-Wallis test, LHβ mRNA; $P < 0.05$ and FSHβ mRNA; $P < 0.0005$). There were age effects on the AP contents of LHβ ($P = 0.0213$) and FSHβ ($P = 0.0001$) mRNAs. This effect was due mainly to the increase with age of both mRNAs seen in FR rats ($P < 0.01$ for LHβ mRNA and $P < 0.05$ for FSHβ mRNA). In
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 (11,36,37,44). By contrast, in old rats, when GH levels of AL rats have fallen, FR sustains youthful pulsatility (44). 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. Although complete age-course studies of circulating levels of GH have not been reported, our GH mRNA data roughly map more completely the action of FR on hypothalamic-releasing factors. 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-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.

![Figure 5](https://academic.oup.com/jn/article-abstract/131/6/1687/4686808)
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 pro lactinoma 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 the only 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 for aged FR rats, they are decreased in aging male F344 rats, a result that parallels our finding of a reduction in the gonadal mRNAs encoding these hormones in AL rats. Although testosterone levels are also decreased in AL F344 rats, progesterone levels are markedly elevated (48–50). Gruenwald and colleagues (49) postulated that the elevated progesterone, through negative feedback at the hypothalamic-pituitary level, underlies the reduced gonadotropin levels of aged F344 rats. Elevated progesterone could also account for the suppression of gonadotropin mRNAs that we observed. Increased progesterone and reduced testosterone in AL F344 rats are believed to be consequences of the Leydig cell tumors that are common in aged F344 rats (48,51). Because FR reduces the incidence of Leydig cell tumors (52), the absence of suppressed gonadotropin mRNAs in old FR rats may reflect reduced testicular tumorigenesis. Why gonadotropin mRNAs in old FR rats are elevated, however, rather than maintained at young values, is less apparent.

In conclusion, the effect of food restriction on expression levels of mRNAs encoding major pituitary tropic hormones is remarkably complex. The changes corroborate many but not all of the changes reported elsewhere in blood levels of hormones the mRNAs encode. Together, these findings point to a highly altered state of endocrine activity in FR rats. That many of the hormonal changes in FR rats correspond to those in mutant mice with extended life span provides added impetus to determine the role if any of altered hormonal status to the extended life of these animal models.

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LITERATURE CITED

nocortin RNA and adrenocorticotropic hormone biosynthesis. J. Gerontol. 50A: B288–B294.