

# Thyroxin Phases the Circadian Fattening Response to Prolactin<sup>1</sup> (34634)

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Diurnal variations in metabolic responses to prolactin have been found in birds (1), lizards (2), frogs (2), and fish (2, 3). On 16-hr photoperiods (L:D = 16:8), midday injections cause increases in fat stores; whereas, early and late injections during the light period are ineffective, or may suppress the level of fat stores. In *Fundulus chrysotus*, midday injections cause increases in fat stores that reach plateau levels of 150 to 400% of the control values after 6 to 9 daily injections (2, 3). Early injections reduce the level of fat stores provided the untreated fish are not already low in fat content. These investigations suggested that the photoperiod times the responsive periods to prolactin through endogenous systems. The present study was undertaken to determine whether or not thyroxin can phase a circadian rhythm of fattening response to prolactin in the fresh water killifish, *Fundulus chrysotus*.

In a series of preliminary experiments using 16-hr photoperiods we discovered that varying the time of thyroxin injections can alter the time of the diurnal fattening response to prolactin. Injections of prolactin within 12 hr after injections of thyroxin tended to suppress fat stores; whereas, injections of prolactin more than 12 hr, but fewer than 24 hr, after injections of thyroxin were stimulatory. The phasing effect of the injected thyroxin appeared to override, in part, the phasing action of the photoperiod on the rhythm of fattening response. In order to remove periodic information supplied by the photoperiod and to eliminate possible distortions to the phasing action by thyroxin, we performed two experiments, reported here, in

which fish were maintained in continuous light.

*Methods.* Adult fish of both sexes about 1 year old and weighing an average of 1.2 g were netted from freshwater ponds near Baton Rouge, Louisiana, in June (Expt. 1, Table I) and July (Expt. 2, Table II), 1969. The fish were maintained in outdoor pools until they were transferred indoors for the experimental period. At the time the experiments were begun, the peak of the breeding season was past but the males were still in breeding colors. Some females contained small immature eggs. The fish were separated into groups of 3 or 4 and placed into aquariums having water depths of 5–6 cm and surface areas of about 550 cm<sup>2</sup>. The aquariums were placed in refrigerated incubators where the temperature was maintained at  $26 \pm 1^\circ$ . Continuous light was supplied by daylight fluorescence with an intensity of 140–170 lx at the water surfaces. Dried food in overabundance and changes in water (aged and adjusted to  $26^\circ$ ) were provided at the times of injection.

In Expt. 1 the fish were placed in continuous light on June 19, 1969. Thyroxin (1  $\mu$ g of L-thyroxin in 0.01 ml of 0.65% saline) was injected intraperitoneally in one-half of the fish at 0600 and in the other half at 1800 hr on June 24 and 26. Each of the two thyroxin-treated groups was injected (ip) subsequently with prolactin (1  $\mu$ g of ovine prolactin in 0.65% saline) or saline at 1200 or 2400 hr on June 28, 29, 30, and July 1. The fish were killed on July 2, 24 hr following each group's final injection. The total body fat, soluble in petroleum ether, was extracted from the dried carcasses using a Soxhlet apparatus. The amount of fat is expressed as a percentage of the dry body weight (dry lipid index).

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TABLE I. Temporal Synergism Between Thyroxin and Prolactin in the Regulation of Body Fat Levels: Exp. 1.<sup>a</sup>

Group	No.	Days 1 <sup>b</sup> and 3		Days 5, 6, 7, and 8		Phase angle <sup>c</sup>	Dry lipid index <sup>c</sup>	Probabilities <sup>d</sup>
		Injection	Time	Injection	Time			
A	6	Thyroxin	0600	Saline	1200	6	8.3 ± 1.1	
B	6	Thyroxin	0600	Saline	2400	18	7.7 ± 1.0	
C	6	Thyroxin	1800	Saline	1200	18	8.2 ± 0.7	
D	3	Thyroxin	1800	Saline	2400	6	6.3 ± 1.5	
E	9	Thyroxin	0600	Prolactin	1200	6	6.6 ± 0.5	vs. F ( $p < .07$ ); vs. G ( $p < .07$ )
F	7	Thyroxin	0600	Prolactin	2400	18	9.6 ± 1.5	vs. E ( $p < .07$ ); vs. H ( $p < .05$ )
G	7	Thyroxin	1800	Prolactin	1200	18	9.0 ± 1.1	vs. E ( $p < .07$ ); vs. H ( $p < .02$ )
H	8	Thyroxin	1800	Prolactin	2400	6	6.0 ± 0.8	vs. F ( $p < .05$ ); vs. G ( $p < .02$ )

<sup>a</sup> *Fundulus chrysolatus* were placed on continuous light 5 days before the first injections.

<sup>b</sup> June 24, 1969.

<sup>c</sup> Percentage lipid of dry body weight.

<sup>d</sup> Student's *t*.

<sup>e</sup> The number of hours from the time of day when thyroxin was injected to the time of day when prolactin was injected.

Expt. 2 was conducted in a similar manner with few differences. The fish were placed in continuous light on July 23, 1969. Thyroxin was injected on July 28 and 29, and prolactin was injected on July 31 and August 1, 2, and 3. The animals were killed for fat analyses on August 4. The regimens of the two experiments differ principally in that saline-

injected controls accompany the groups treated with thyroxin in Expt. 2 rather than the groups treated with prolactin, as in Expt. 1. The experimental conditions of the two experiments are defined further in Tables I and II.

**Results.** In both experiments it is apparent that the phase angle between the time of

TABLE II. Temporal Synergism Between Thyroxin and Prolactin in the Regulation of Body Fat Levels: Exp. 2.<sup>a</sup>

Group	No.	Days 1 <sup>b</sup> and 2		Days 4, 5, 6, and 7		Phase angle <sup>c</sup>	Dry lipid index <sup>c</sup>	Probabilities <sup>d</sup>
		Injection	Time	Injection	Time			
A	8	Saline	0600	Prolactin	1200		5.8 ± 1.1	
B	7	Saline	0600	Prolactin	2400		5.3 ± 0.6	vs. D ( $p < .02$ )
C	6	Thyroxin	0600	Prolactin	1200	6	5.7 ± 0.6	vs. D ( $p < .05$ ); vs. G ( $p < .05$ )
D	8	Thyroxin	0600	Prolactin	2400	18	7.8 ± 0.6	vs. B ( $p < .02$ ); vs. C ( $p < .05$ ); vs. H ( $p < .01$ )
E	5	Saline	1800	Prolactin	1200		5.5 ± 0.5	vs. G ( $p < .02$ )
F	7	Saline	1800	Prolactin	2400		6.1 ± 0.5	
G	8	Thyroxin	1800	Prolactin	1200	18	8.3 ± 0.9	vs. C ( $p < .05$ ); vs. E ( $p < .02$ ); vs. H ( $p < .01$ )
H	7	Thyroxin	1800	Prolactin	2400	6	5.1 ± 0.5	vs. D ( $p < .01$ ); vs. G ( $p < .01$ )

<sup>a</sup> *Fundulus chrysolatus* were placed on continuous light 5 days before the first injections.

<sup>b</sup> July 28, 1969.

<sup>c</sup> Percentage lipid of dry body weight.

<sup>d</sup> Student's *t*.

<sup>e</sup> The number of hours from the time of day when thyroxin was injected to the time of day when prolactin was injected.

thyroxin injection and the time of prolactin injection is of primary importance. When prolactin injections follow the time of thyroxin pretreatment by 18 hr there is an increase in total fat stores (Tables I and II). In the two experiments, it is possible to make 8 comparisons between groups receiving prolactin 6 hr after the time of thyroxin pretreatment and those receiving prolactin 18 hr after the time of thyroxin pretreatment. In every comparison, the groups receiving prolactin at 18 hr are higher than those receiving prolactin at 6 hr. Student's *t* test was used for the statistical analyses (Tables I and II). The real time of day when the injections were made seems to be irrelevant. The lack of differences among the prolactin controls (Expt. 1, Table I) as well as among the thyroxin controls (Expt. 2 Table II) indicate that the hormones, rather than handling, are responsible for the effects noted.

Following the breeding season in May and June, the fat content of *F. chrysotus* declines. The inability to demonstrate significant losses of fat stores in the fish receiving injections of prolactin 6 hr following the time of thyroxin pretreatment may be a result of a lower limit of fat content found in the untreated fish beyond which there can be no further losses. Prolactin produces diurnal losses in fat stores when the dry lipid index of control fish is greater than 10 (2, 3, and unpublished data).

**Discussion.** These results suggest that the regulation of fat stores in a fish involves a synergistic relationship between thyroxin and prolactin. A synergism of thyroxin and prolactin with regard to fattening was also reported in pigeons (4). The synergism, however, is a special one in *F. chrysotus* that involves a temporal relationship.

An adequate explanation of our results seems to reside in considerations of circadian periodicities. Thyroxin appears to "set the clock" of the systems involved in fattening. Prolactin triggers the fattening response if it is present during a sensitive period timed by thyroxin. Once the "clock" is set by thyroxin the sensitive period recycles in a circadian manner under conditions of continuous light. Thus, in brief, the regulation of fat storage

may be visualized as a function of the phase angle between a pulse of thyroxin and the diurnal increase in levels of prolactin (Fig. 1).

The persistence for days of a circadian cycle of fattening sensitivity following thyroxin administration indicates that thyroxin acts as a signal to set a "biological time clock." It does not appear to maintain the rhythm by direct action on the biochemical milieu of the fat cells. Conceivably, thyroxin may phase the rhythm of another regulatory system more directly involved in fat metabolism; one that is capable of prolonged independent oscillation.

Although similarities exist between the phasing effect of thyroxin and that of the photoperiod, further research is required to ascertain whether the one is causally related to the other. The anterior pituitary and the pineal gland could relay photoperiodic information to the thyroid. Thyroid-stimulating hormone may be expected to provide a periodic stimulatory effect (5, 6), whereas melatonin could have a periodic inhibitory effect on thyroxin release (7).

Because diurnal fattening responses to prolactin have been observed in a variety of vertebrates (1-3), it seems likely that certain aspects of the mechanism in *F. chrysotus* may be operative also in the regulation of fat stores in other animals. Diurnal rhythms of serum and pituitary thyroid stimulating hormone (5, 6) as well as of pituitary prolactin (8, 9) have been demonstrated in mammals. The functional significance of diurnal

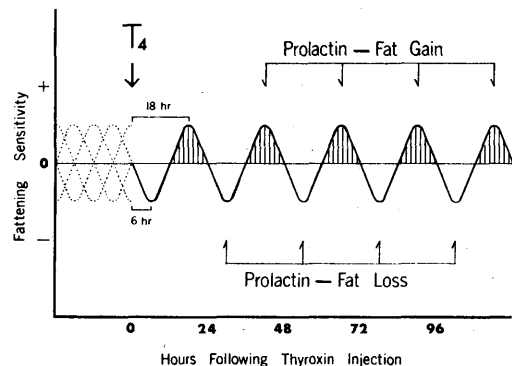


FIG. 1. Synchronization of the circadian fattening response to prolactin by thyroxin ( $T_4$ ): A model.

rhythms of prolactin levels is apparent in a migratory sparrow, *Zonotrichia albicollis*, in which the diurnal rhythm of pituitary content was found to have different phase relations with the photoperiod at two seasons of the year (10). During the spring migratory period when the bird is fat, prolactin is released during the afternoon. In August when the bird is lean, prolactin is released between midnight and sunrise. These findings correlate well with other observations in *Z. albicollis* that injections of prolactin at midday cause fattening; whereas, injections early in the day result in losses of fat stores (1).

Alternatives to the thyroxin-prolactin interrelationship for regulation of fat stores are to be expected. For example, luteinizing hormone (LH) might replace prolactin in regulating some types of fattening. LH is a potent stimulator of fat deposition in *F. chrysotus* (2, 3), frogs (*Rana pipiens*), and lizards (*Anolis carolinensis*) (unpublished data), and possibly birds (*Zonotrichia leucophrys gambelii*) (11), provided the injections are given at a specific time during the day.

The finding of a temporal synergism between thyroxin and prolactin in the control of body fat levels poses many interesting questions. Pursuit of these and other questions raised by an expanding literature dealing with circadian rhythms [for reviews (12, 13)] contributes to our understanding of endocrine regulation of physiological processes.

*Summary.* The diurnal fattening response to prolactin in the Golden Topminnow, *Fundulus chrysotus*, involves a circadian periodicity of fattening sensitivity to the hormone. Phasing of the rhythm in fish kept in continuous light can be obtained by injections of thyroxin. A phase angle of 18 hr between the

time of thyroxin injection and the time of prolactin injection results in conditions favoring the accumulation of fat stores. Phase angles of 6 hr do not promote fattening.

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