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Effects of Starvation and Refeeding on Gonadotropin and Thyrotropin Subunit mRNAs in Male Japanese Quail

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ABSTRACT—The contents of mRNAs encoding LHβ-, FSHβ-, TSHβ- and common α-subunit precursor molecules were measured in food-deprived and subsequently re-fed male Japanese quail. Pituitary LHβ, FSHβ and common α mRNA levels were decreased by starvation, and increased to the control levels by re-feeding. The rates of decreases of LHβ and common α mRNA levels were greater the corresponding rate for FSHβ levels. Pituitary TSHβ mRNA levels were not decreased by starvation, but increased transitorily by re-feeding. Plasma LH and triiodothyronine levels were decreased by starvation, and then increased to control levels by re-feeding, while plasma FSH and thyroxine levels did not show significant changes. Plasma LH and FSH levels showed positive correlations with pituitary common α and FSHβ mRNA levels, respectively, while plasma thyroxine levels showed a negative correlation with TSHβ mRNA levels. Hepatic weight was decreased slightly but significantly by starvation, and then showed a remarkable rebound after re-feeding was started. These results suggest that LH synthesis and secretion are more sensitive to starvation than FSH synthesis and secretion in Japanese quail, and that LH production recovered to initial levels within several days when birds were fully fed. Also, there is a possibility that the synthesis of TSH is accelerated transitorily by re-feeding. Furthermore, these results showed that there are different relationships between the plasma levels of LH, FSH, and TSH and the various hormone subunit mRNA levels. The remarkable change in hepatic weight leads us to assume that hepatic thyroid hormone metabolism is affected by starvation and re-feeding.

Key words: starvation, refeeding, gonadotropin, thyrotropin, mRNA

INTRODUCTION

In birds, food availability is one of the major environmental factors that affect functions of endocrine systems such as the hypophysio-gonadal, -thyroidal and -adrenocortical systems (Scanes and Griminger, 1990). It is well known that starvation affects reproductive activity in various vertebrates including birds (Hosoda *et al*., 1955; Morris and Nalbandov, 1961; Vigersky *et al*., 1977; Brake and Thaxton, 1979; Glass and Swerdloff, 1980; Hoffer *et al*., 1986). It is also known that the suppressive effect of starvation on reproduction is mediated by a reduction of gonadotropin secretion (Negro-Vilar *et al*., 1971; Root and Russ, 1972; Howland and Skinner, 1973; Stewart *et al*., 1973; Howland, 1975; Scanes *et al*., 1976; Campbell *et al*., 1977; Tanabe *et al*., 1981; Badger *et al*., 1985; Hoshino *et al*., 1988; Foster *et al*., 1989; Xie, 1991), and that gonadotropin secretion increases again during re-feeding (Howland, 1975; Tanabe *et al*., 1981; Hoshino *et al*., 1988; Xie, 1991).

Recently, the interests of endocrinologists have been focused on the expression of the genes encoding the gonadotropin subunit precursor molecules. Some investigators have studied changes in intra-pituitary contents of mRNAs for the subunit precursor molecules in the rat (Bergendahl *et al*., 1989, 1991; Bergendahl and Huhtaniemi, 1993), sheep (Thomas *et al*., 1990) and Japanese quail (Kobayashi and Ishii, 2002). From these results, these investigators suggested that starvation affects reproductive activity by not only stopping the secretion of the gonadotropins but also by suppressing the transcription of the hormone subunit precursor genes. However, changes in mRNAs for the gonadotropin subunit precursor molecules during the recovery phase

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from starvation have never been reported in any vertebrate species as far as we are aware.

Birds, unlike mammals, need continuous feeding and hence are expected to be extremely sensitive to starvation. Starvation can be caused accidentally by changes in natural environmental conditions such as storms, floods, and snow

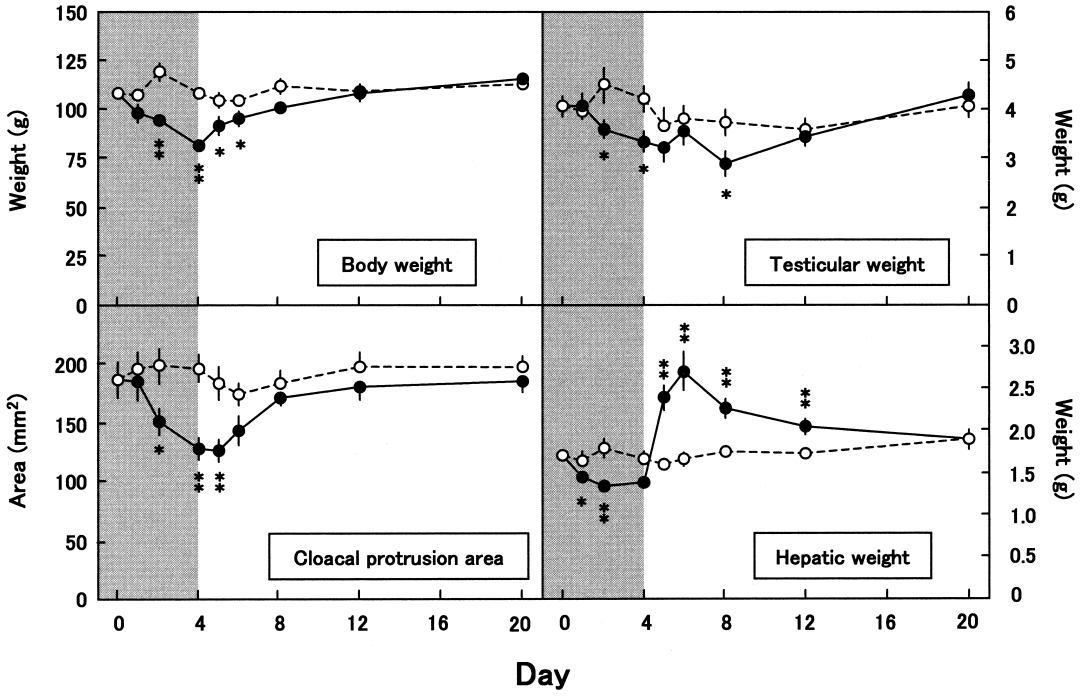

Fig. 1. Changes in the body weight, testicular weight, cloacal protrusion area and hepatic weight in experimental (solid circles with solid line) and control (open circle with broken line) groups. Circles and bars represent the mean±SEM. Shaded area represents the food deprivation period. Significant differences between the experimental and control groups on the same day are indicated at $P<0.05$ (*) and $P<0.01$ (**).

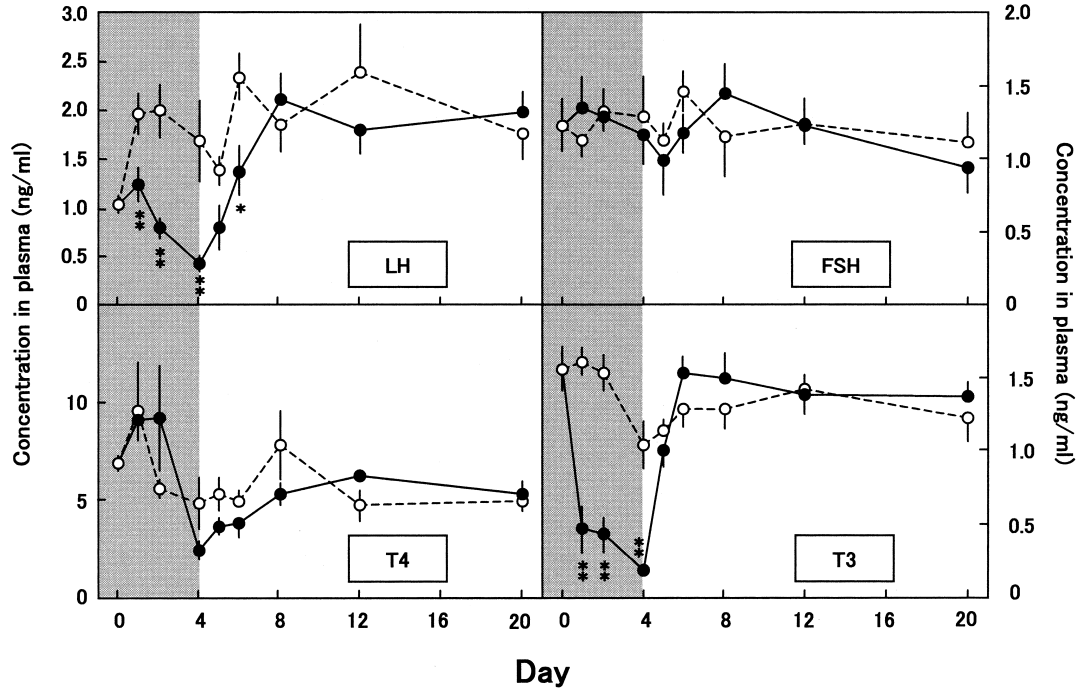

Fig. 2. Changes in plasma concentrations of luteinizing hormone (LH), follicle-stimulating hormone (FSH), thyroxine (T4) and triiodothyronine (T3) in experimental (solid circles with solid line) and control (open circle with broken line) groups. Circles and bars represent the mean±SEM. Shaded area represents the food deprivation period. Significant differences between the experimental and control groups on the same day are indicated at $P<0.05$ (*) and $P<0.01$ (**).

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in feral birds, and by husbandry problems in domestic and experimental birds. Furthermore, temporary food deprivation is used as a practical management technique for forced molting followed by an increase in egg production after the re-feeding in the hen. The resumption of egg-laying is accompanied by a recovery of plasma luteinizing hormone (LH) levels from the suppressed conditions of starvation (Tanabe *et al*., 1981; Hoshino *et al*., 1988). Therefore, the study of changes in pituitary contents of mRNA encoding gonadotropin subunit precursor molecules after the re-feeding is important for both veterinary and ecological endocrinology.

Furthermore, starvation and re-feeding are accompanied by changes in metabolic states. The metabolic state is correlated with the thyroid hormone secretion which is also

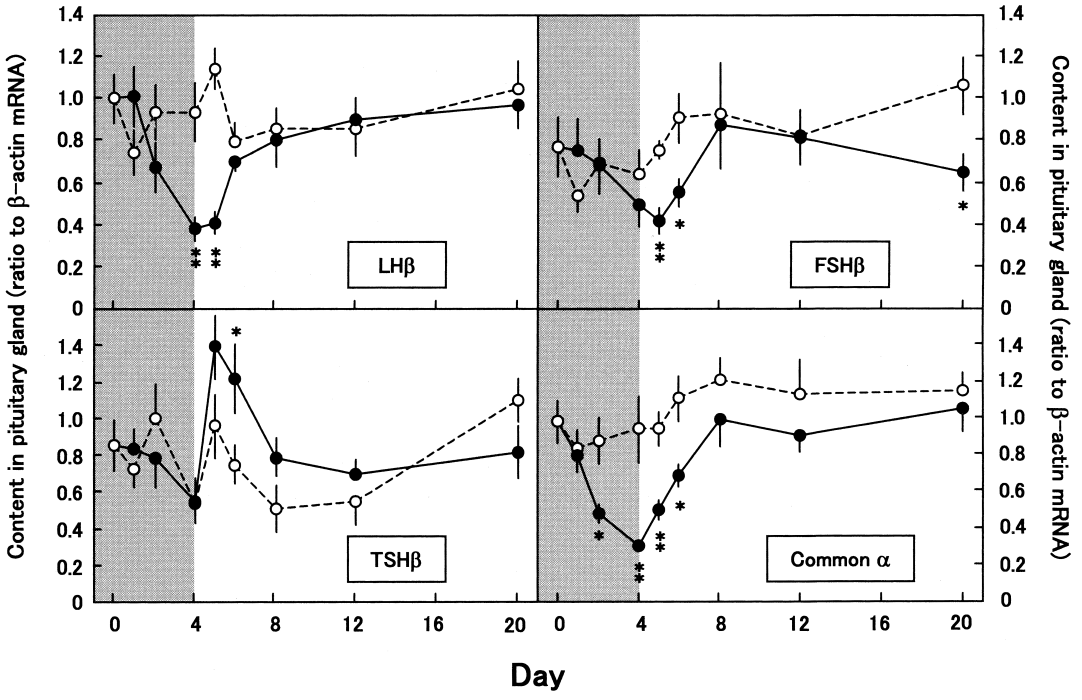

Fig. 3. Changes in pituitary contents of LHβ, FSHβ, TSHβ and common α mRNA in experimental (solid circles with solid line) and control (open circle with broken line) groups. The values are expressed as the ratio to β-actin mRNA content. Circles and bars represent the mean±SEM. Shaded area represents the food deprivation period. Significant differences between the experimental and control groups on the same day are indicated at P<0.05 (*) and P<0.01 (**).

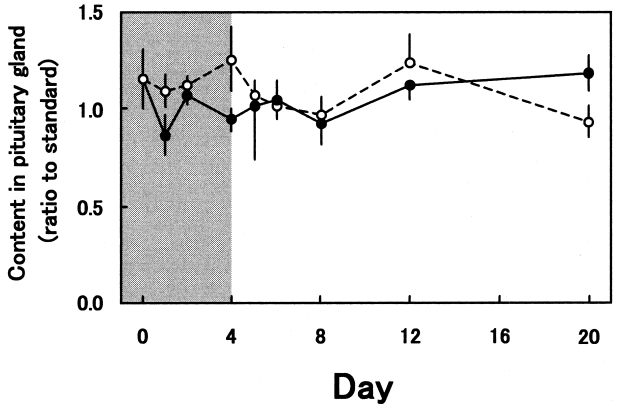

Fig. 4. Changes in pituitary contents of β-actin mRNA in experimental (solid circles with solid line) and control (open circle with broken line) groups. The contents are expressed as the ratio to a standard quail pituitary RNA preparation (see Materials and Methods). Circles and bars represent the mean±SEM. Shaded area represents the food deprivation period.

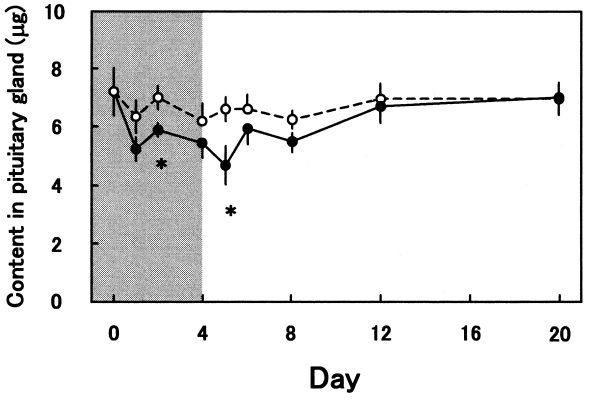

Fig. 5. Changes in pituitary contents of total RNA in experimental (solid circles with solid line) and control (open circle with broken line) groups. Circles and bars represent the mean±SEM. Shaded area represents the food deprivation period. Significant differences between the experimental and control groups on the same day are indicated at P<0.05 (*).

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reported to be influenced by starvation in birds (May, 1978; Klandorf and Harvey, 1985; Hoshino *et al*., 1988; Geris *et al*., 1999; Van der Geyten *et al*., 1999; Kobayashi and Ishii, 2002). We reported that starvation decreased the content of mRNA encoding the thyroid stimulating hormone (TSH) β-subunit precursor molecule in the Japanese quail (Kobayashi and Ishii, 2002). The results of this study suggested that starvation also suppresses TSH synthesis in the pituitary gland in birds. Accordingly, it is important to measure mRNA encoding TSH β-subunit precursor molecule as well as follicle-stimulating hormone (FSH) and LH subunit precursor molecules for understanding the relations between each hormone subunit mRNA levels.

We therefore studied changes in pituitary contents of mRNAs encoding precursor molecules of the β-subunits of FSH, LH and TSH and the common α-subunit in male Japanese quail (*Coturnix coturnix japonica*) during the course of starvation and its recovery phase during re-feeding.

MATERIALS AND METHODS

Birds and treatments

Ten-week old male Japanese quail were purchased from a commercial source (Tokai Yuki, Aichi Prefecture, Japan). Birds were kept in individual cages under constant conditions of illumination (16hr light : 8hr darkness) and temperature (25°C) and provided with food and water *ad libitum* for a month. Birds were divided into 8 experimental and 9 control groups randomly. Each group consisted of 8 to 12 birds. Birds of the experimental group were deprived of food for 4 days and then fed subsequently for 16 days. We limited the duration of starvation to 4 days in order to minimize pain of the experimental birds. Drinking water was provided *ad libitum* throughout the 20 days. Birds of the control group were continuously provided with food and water *ad libitum*. Birds of the experimental groups were sacrificed 1, 2 and 4 days after the start of food deprivation and 1, 2, 4, 8 and 16 days after the start of feeding. The sacrifice was done according to the Regulation of the Ministry of Education, Science and Culture for the experimental animals. Birds of the control groups were sacrificed on the 0 day and then on the same time schedule as the experimental groups. Body weight and size of the cloacal protrusion were measured at the time of sacrifice. Trunk blood was collected just before sacrifice, and plasma was separated by centrifugation. Pituitaries were excised within 3 min after sacrifice and immediately frozen in liquid nitrogen. Testes and liver were dissected out and weighed. The frozen pituitaries were stored at –80°C and plasma samples were stored at –24°C.

RNA extraction

Total RNA was extracted from each pituitary gland using a commercial kit (ISOGEN, NIPPON GENE, Tokyo, Japan). Extracted RNA was dissolved in diethyl pyrocarbonate treated dH_2O, and stored at –80°C until electrophoresis. Total RNA concentration was determined by measuring the optic absorbance at 260nm.

Hybridization probes

To estimate contents of gonadotropin and thyrotropin subunit mRNAs by Northern blotting, the following cDNAs were used as hybridization probes: luteinizing hormone β-subunit (LHβ) cDNA (pQL119 by Ando and Ishii, 1994), a *Hind*III-digested 5' fragment of follicle-stimulating hormone β-subunit (FSHβ) cDNA (pQF611 by Kikuchi *et al*., 1998), thyroid stimulating hormone β-subunit (TSHβ) cDNA (pQT11 by Kikuchi and Ishii, unpublished), pituitary glycoprotein hormone α-subunit (common α) cDNA (pQA312 by Ando and

Table 1. Simple and partial correlation analyses between plasma hormone levels and its corresponding hormone subunit mRNA level for the control and experimental groups.

Dependent variable	Independent variable	r in simple correlation (P)	r in partial correlation (P)	Fixed variable in partial correlation
Control group				
LH	LHβ	0.0652 (0.5812)	0.0135 (0.9100)	common α
LH	common α	0.3325 (0.0038)**	0.3269 (0.0048)**	LHβ
FSH	FSHβ	0.5287 (<0.0001)***	0.5336 (<0.0001)***	common α
FSH	common α	0.1449 (0.2182)	–0.1674 (0.1569)	FSHβ
T4	TSHβ	–0.3439 (0.0031)**	–0.3513 (0.0026)**	common α
T4	common α	–0.1321 (0.2687)	–0.1527 (0.2035)	TSHβ
T3	TSHβ	0.1048 (0.3775)	0.1061 (0.3752)	common α
T3	common α	0.0310 (0.7949)	0.0350 (0.7707)	TSHβ
Experimental group				
LH	LHβ	0.4353 (0.0001)***	0.1239 (0.2862)	common α
LH	common α	0.6544 (<0.0001)***	0.5526 (<0.0001)***	LHβ
FSH	FSHβ	0.5591 (<0.0001)***	0.6210 (<0.0001)***	common α
FSH	common α	–0.0361 (0.7550)	–0.3278 (0.0038)**	FSHβ
T4	TSHβ	–0.3607 (0.0017)**	–0.3736 (0.0012)**	common α
T4	common α	0.2365 (0.0440)*	0.2573 (0.0291)*	TSHβ
T3	TSHβ	0.1969 (0.0927)	0.2006 (0.0889)	common α
T3	common α	0.2528 (0.0298)*	0.2555 (0.0291)*	TSHβ

Note. Plasma hormone levels were taken as dependent variables and mRNA levels as independent or fixed variables. Hormone subunit mRNA levels were expressed with ratio to β-actin mRNA. Significant correlation at P<0.05 (*), P<0.01 (**) and P<0.001(***).

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Ishii, 1994), and chicken β-actin cDNA (Oncor Inc., Gaithersburg, MD, U. S. A.). All these cDNAs were labeled with [α–^{32}P] dCTP (AA0005, Amersham Pharmacia Biotech, UK) using the random prime labeling system (*redi*prime DNA labeling system, Amersham Pharmacia Biotech) followed by purification on a column (ProbeQuant G-50 Micro Columns, Amersham Pharmacia Biotech).

Northern blotting

Contents of gonadotropin and thyrotropin subunit and β-actin mRNAs were measured by Northern blotting as described by Kobayashi and Ishii (2002). Briefly, total RNA samples were electrophoresed and then transferred to nylon membranes (NYTRAN, Schleicher & Schell, Postfach, Germany). As the internal reference standard, a total RNA sample extracted from pituitaries of a number of normal sexually active adult males of the Japanese quail was applied to every plate and electrophoresed. Hybridization signals on the membranes were analyzed and quantified in a BAS-2000 II Bio-Imaging analyzer (Fuji Photo Film Co., Ltd., Japan). After signal quantification, labeled probe on membranes was removed and then hybridizations with the other probes were performed. Hybridization signals for all the mRNAs were expressed as relative values to the signal of the reference standard in each electrophoretic run and were standardized among different electrophoresis plates. Contents of the gonadotropin and thyrotropin subunit mRNAs were expressed as ratios of the values of the hormone subunit precursors to the values of β-actin mRNA in the pituitary gland.

Hormone assays

Plasma concentrations of LH and FSH were measured by double-antibody RIA for chicken LH and FSH as described originally by Hattori and Wakabayashi (1979) for LH and Sakai and Ishii (1983) for FSH with modifications (Silverin *et al*., 1999). Plasma concentrations of thyroxine (T4) and triiodothyronine (T3) were measured by enzyme immunoassay using a commercial kit (AIA-600, Tosoh Co., Ltd., Japan).

Statistical analyses

Two way analysis of variance was used for statistical analysis. If there was a significant difference between two groups, a Student's t-test or Mann-Whitney U test was applied for comparison of means between two groups on the same day. Relationships between plasma hormone levels and pituitary hormone subunit

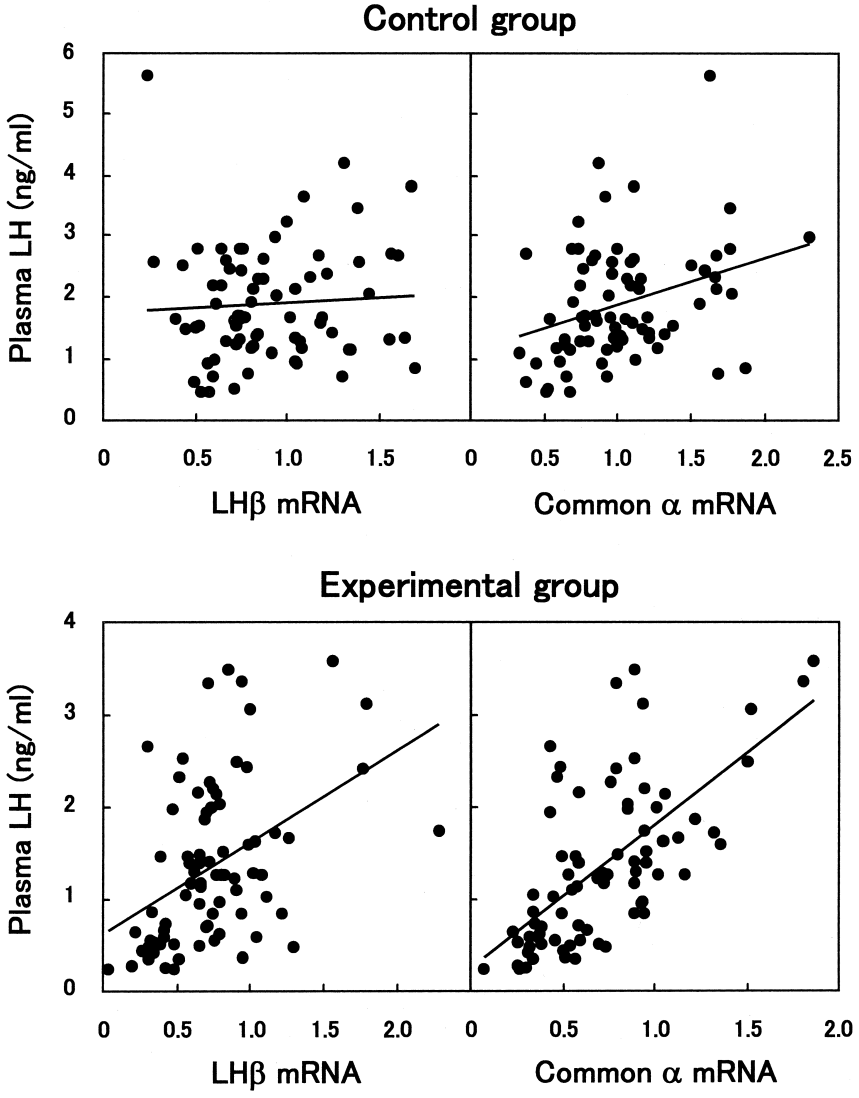

Fig. 6. Correlations between plasma concentrations of LH and pituitary contents of LHβ mRNA and common α mRNA in experimental and control group. Solid line represents regression line.

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mRNA levels were analyzed by simple, partial and multiple correlations. A P value less than 0.05 was chosen to be statistically significant.

RESULTS

Weights of the body, testes and liver and size of cloacal protrusion

Body and testicular weights and cloacal protrusion area all decreased during the food-deprivation period and increased to initial levels during the re-feeding period, although the rate of the decrease and the recovery speed differed (Fig. 1). In contrast, the hepatic weight showed a different pattern of the change. It decreased slightly but significantly during the food deprivation period, but during the first two days of the re-feeding period it rapidly increased up to about 160% of the initial level (Fig. 1). The hepatic weight then decreased to the initial level by the end of the re-feeding period.

Plasma concentrations of gonadotropins and thyroid hormones

The mean plasma LH level in the experimental group showed a statistically significant decrease during the food-deprivation period and then increased to the control level 4 days after the re-feeding was started, while the plasma FSH level in the experimental group as well as that in the control group randomly fluctuated around the initial normal level (Fig. 2). The mean plasma LH level at the onset of starvation was relatively low, but after a day the level in control birds showed a level similar to the other control birds. The mean plasma T3 level in the experimental group also decreased during the food-deprivation period and returned to the initial level as soon as the re-feeding was started (Fig. 2). The rates of the T3 decrease during the food-deprivation period and of the T3 increase during the re-feeding period were more drastic than corresponding rates for the plasma LH level.

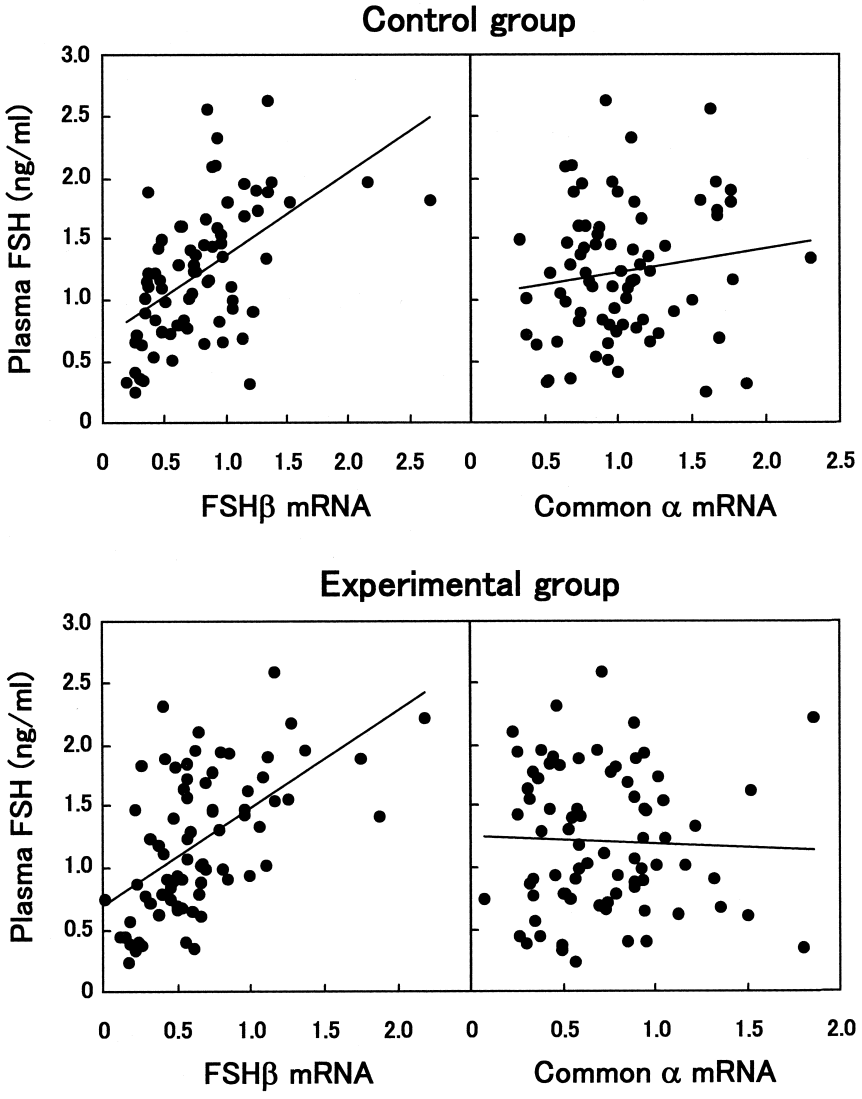

Fig. 7. Correlations between plasma concentrations of FSH and pituitary contents of FSHβ mRNA and common α mRNA in experimental and control group. Solid line represents regression line.

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The mean plasma T4 level in the experimental group decreased slightly by the end of the food-deprivation period, and then increased to the initial level through the initial half of the re-feeding period, and these changes were statistically insignificant (Fig. 2). Also, the range of individual variation was rather wide in the plasma T4 level and these changes were statistically insignificant when compared with the control levels.

LHβ, FSHβ, TSHβ and common α mRNA pituitary contents

The mean LHβ, FSHβ and common α subunit mRNA levels expressed as the ratio to the β-actin mRNA content in the experimental group were decreased by starvation, and statistically significant differences from control levels were observed at or after the end of the food-deprivation period in the LHβ and FSHβ subunit mRNAs (Fig. 3). The mean common α mRNA level was decreased more rapidly by starvation and a statistically significant difference from the control level was observed 2 days after the start of the food deprivation. The suppressed mRNA levels of these subunits in the experimental group increased to the normal (initial) levels after re-feeding was started. However, the mean TSHβ mRNA level showed a small and statistically insignificant decrease during the food deprivation period and then a statistically significant and transitory increase immediately the re-feeding was started (Fig. 3).

β-actin mRNA and total RNA pituitary contents

The mean β-actin mRNA content varied randomly in a small range during the food deprivation and re-feeding periods in both experimental and control groups (Fig. 4). No significant difference was detected between the control and experimental groups throughout the experimental periods.

The mean pituitary content of total RNA in the experimental group always showed a lower level than that in the

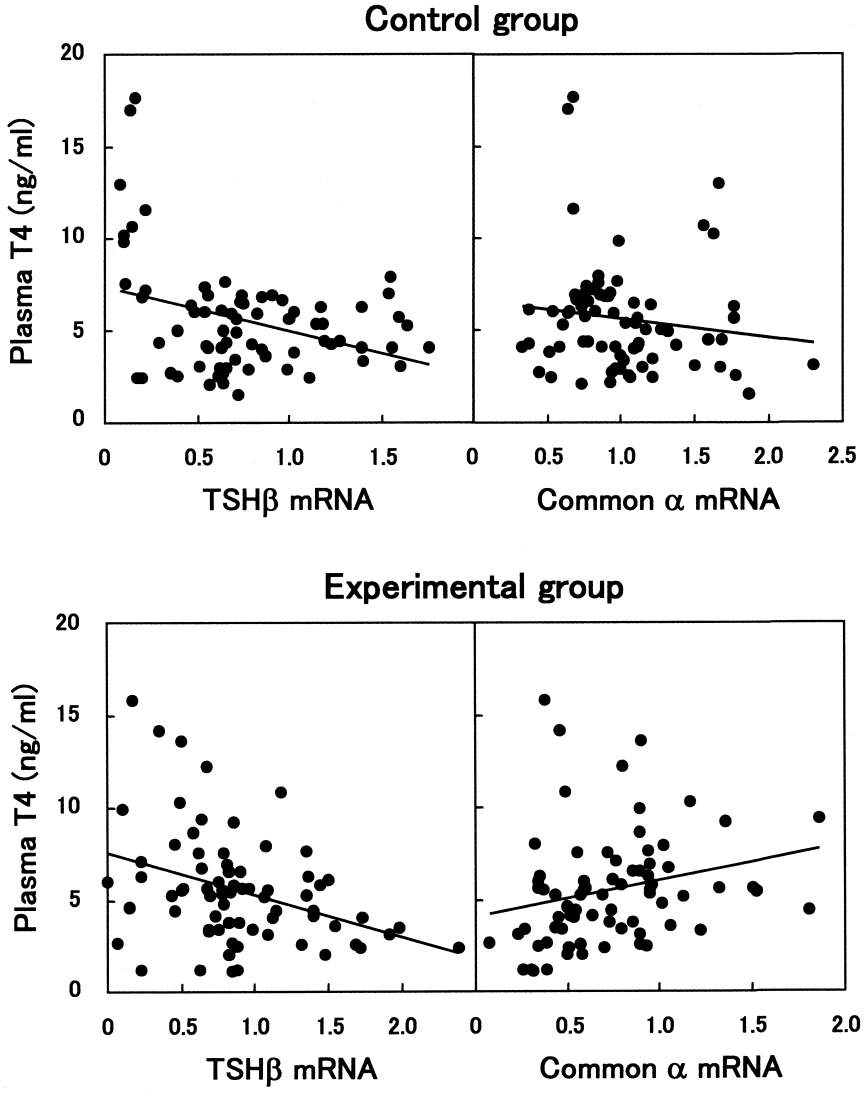

Fig. 8. Correlations between plasma concentrations of T4 and pituitary contents of TSHβ mRNA and common α mRNA in experimental and control group. Solid line represents regression line.

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control level except for the initial and last days of the experimental period (Fig. 5). The difference was statistically significant 2 days after the start of food deprivation and a day after the start of re-feeding.

Correlation analyses

As we measured all the hormone and mRNA parameters in individual animals, we could study relationships between each plasma hormone level and its pertinent hormone subunit mRNA levels by using simple, multiple and partial correlation analyses. The analyses were performed with control and experimental animals separately.

Simple correlation

Scatter diagrams of plasma hormone levels and hormone subunit mRNA levels are shown in Fig. 6, 7, 8 and 9, and correlation coefficients in a simple correlation analysis are shown in Table 1.

In the control group, plasma LH levels showed no correlation with LHβ mRNA levels, but they showed a statistically significant positive correlation with common α mRNA levels (Fig. 6 and Table 1). However, in the experimental group, plasma LH levels showed statistically significant positive correlations with both pituitary LHβ mRNA levels and common α mRNA levels (Fig. 6 and Table 1). On the other hand, plasma FSH levels showed a statistically significant positive correlation with FSHβ mRNA levels but no statistically significant correlation with common α mRNA levels in both the control and experimental groups (Fig. 7 and Table 1). Plasma T4 levels showed a statistically significant negative correlation with pituitary TSHβ mRNA levels but no statistically significant correlation with common α mRNA levels in the control group (Fig. 8 and Table 1). However, in the experimental group, plasma T4 levels showed a statistically significant negative correlation with pituitary TSHβ mRNA levels but a positive correlation with pituitary common α

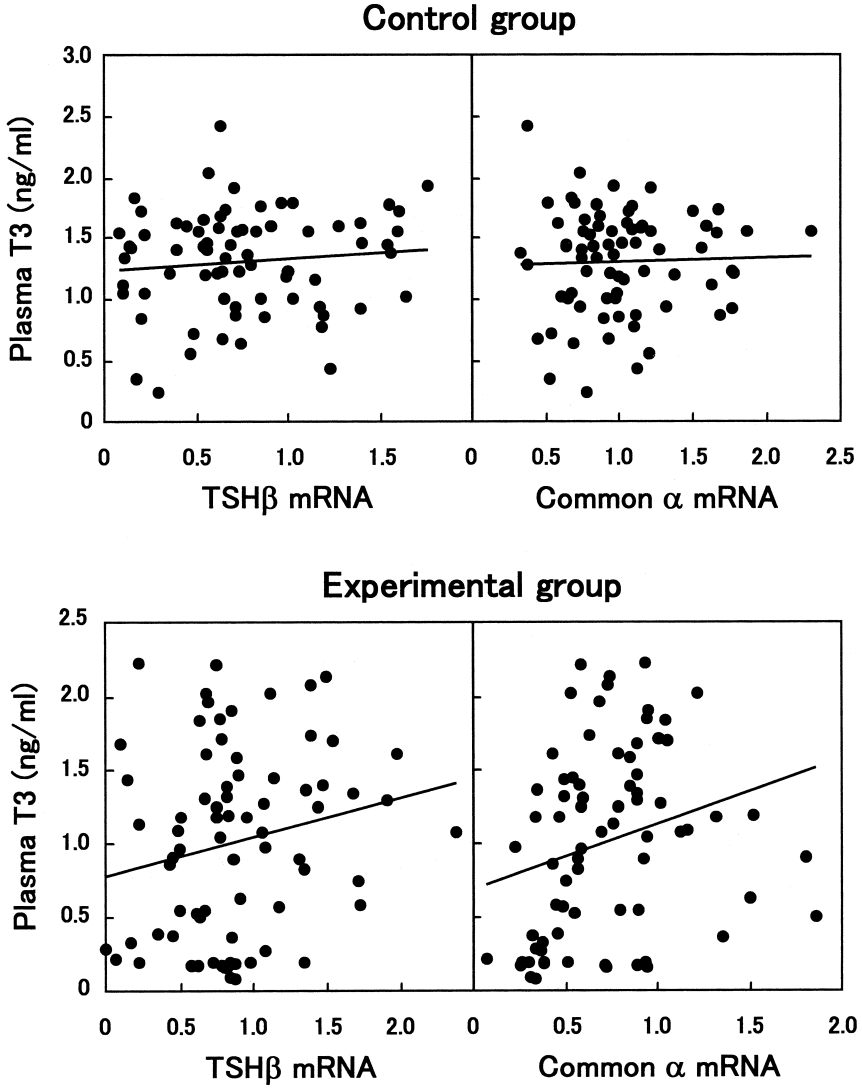

Fig. 9. Correlations between plasma concentrations of T3 and pituitary contents of TSHβ mRNA and common α mRNA in experimental and control group. Solid line represents regression line.

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Table 2. Multiple correlation analyses between plasma hormone levels and its corresponding hormone subunit mRNA levels for the control groups and experimental groups.

Dependent variable	Independent variables	r in multiple correlation (P)
Control group		
LH	LHβ and common α	0.3327 (0.0154)*
FSH	FSHβ and common α	0.5475 (<0.0001)***
T4	TSHβ and common α	0.3726 (0.0058)**
T3	TSHβ and common α	0.1104 (0.6506)
Experimental group		
LH	LHβ and common α	0.6611 (<0.0001)***
FSH	FSHβ and common α	0.6217 (<0.0001)***
T4	TSHβ and common α	0.4332 (0.0007)***
T3	TSHβ and common α	0.3186 (0.0224)*

Note. Plasma hormone levels were taken as dependent variables and mRNA levels as independent variables. Hormone subunit mRNA levels were expressed with ratio to β-actin mRNA. Significant correlation at P<0.05 (*), P<0.01 (**) and P<0.001(***).

mRNA levels (Fig. 8 and Table 1). Plasma T3 levels did not show statistically significant correlations with pituitary TSHβ mRNA levels in both the control and experimental groups. Plasma T3 levels were not correlated with pituitary common α mRNA levels in the control group, but were significantly positively correlated in the experimental group (Fig. 9 and Table 1).

Multiple and partial correlations

To analyze the combined relationship of hormone-specific β-subunit mRNA and common α mRNA levels with plasma hormone levels, we calculated multiple correlation coefficients taking two subunit mRNA levels as the independent variables and a plasma hormone level as the dependent variable. Plasma LH levels showed a statistically significant correlation with LHβ mRNA levels and common α mRNA levels in both the control and experimental groups, and plasma FSH levels also did the same with FSHβ mRNA levels and common α mRNA levels in both the control and experimental groups (Table 2). Plasma T4 levels showed significant correlations with TSHβ mRNA and common α mRNA levels in both control and experimental groups. Plasma T3 levels showed no correlation with TSHβ mRNA and common α mRNA levels in the control group, but showed a significant correlation with TSHβ mRNA and common α mRNA levels in the experimental group (Table 2).

Correlations between levels of each hormone and the pertinent two mRNA levels were more precisely analyzed by fixing (or excluding) the effect of one of the mRNA levels by using partial correlation analysis. In the control group, the results of partial correlation analyses were principally the same as those of simple correlation analyses: plasma LH levels were significantly and positively correlated with the common a mRNA levels but not with LHβ mRNA levels, plasma FSH levels were significantly and positively correlated with FSHβ mRNA levels but not or even slightly-negatively correlated with the common α mRNA levels, plasma T4 levels were significantly and negatively correlated with TSHβ mRNA levels but not or even slightly-negatively correlated with the common α mRNA levels, and plasma T3 levels were insignificantly correlated with both TSHβ mRNA levels and common α mRNA levels (Table 1).

In the experimental group, correlations became clearer than those in the control group except for the correlation of plasma LH levels that was similar to that in the control group (Table 1). Plasma FSH levels became more strongly and positively correlated with FSHβ mRNA levels and more strongly negatively correlated with common a mRNA levels (the negative correlation was statistically significant), plasma T4 levels were significantly negatively correlated with TSHβ mRNA levels as they were in the control group and significantly positively correlated with common α mRNA levels, and plasma T3 levels that showed no significant correlation with common α mRNA levels in the control group became significantly and positively correlated with common α mRNA levels in the experimental group (Table 1).

DISCUSSION

In the present study, we found that testicular and hepatic weights and cloacal protrusion area decreased during the food-deprivation period and that testicular weight and cloacal protrusion area returned to normal during the re-feeding period. These results are well explained by the metabolic and endocrine effects of starvation. However, the hepatic weight showed a remarkable overshoot or rebound during the re-feeding period. A similar result has been reported in the chicken (Kita *et al*., 1993). Such a transient increase in the hepatic weight cannot be explained with the hormonal parameters we measured in the present study. An unknown humoral factor would be needed to explain this overshoot in the hepatic weight, and a candidate for the factor will be discussed later.

Recently, we have reported that starvation decreased

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levels of FSH and LH in plasma and FSHβ, LHβ and common α mRNA levels in the pituitary gland in male Japanese quail (Kobayashi and Ishii, 2002). In the present study, we confirmed the previous results except for the change in the plasma FSH level. The plasma FSH level in the experimental group did not show a significant decrease during 4 days of starvation in the present study, while it decreased significantly during the shorter starvation period of 3 days in the previous study (Kobayashi and Ishii, 2002). Similar results to the present one have been reported in the chicken and rat. In the chicken, Scanes *et al*. (1976) reported that both plasma LH and FSH were decreased by starvation but for the full response LH required 12 hr and FSH 48 hr. In the rat, Xie (1991) noted that the decrease in plasma FSH was less remarkable than the decrease in plasma LH. Howland (1975) also reported that food restriction did not influence plasma FSH but decreased plasma LH significantly. These results are consistent with our present results. However, we found in both the previous and present studies that the FSHβ mRNA level in the pituitary gland was decreased by starvation. Furthermore, in the present study, the decrease became statistically significant after the end of the food-deprivation, i.e. one and two days after the re-feeding was started. Accordingly, we may conclude that the plasma FSH level decreases more slowly than the plasma LH level under starvation. The difference in the plasma FSH level results between the present and previous studies of us may be explained by assuming difference in the sensitivity to starvation in experimental birds due to differences in the nutritional condition of the birds.

All of the present and previous studies by us and the other investigators indicate that FSH and LH secretion are influenced in a different way by starvation, i.e. LH secretion is more sensitively suppressed by starvation than FSH secretion. A similar differential effect was observed at the pituitary FSHβ and LHβ mRNA levels in the present study. The level of LHβ mRNA was decreased by starvation at a higher rate than the level of FSHβ mRNA.

Furthermore, correlation analyses in the present study showed that the plasma LH level was solely and positively correlated with the pituitary common α mRNA level and not with the pituitary LHβ mRNA level, while the plasma FSH level was positively correlated with the pituitary FSHβ mRNA level and not (in the simple correlation) or even negatively correlated (in the partial correlation) with the pituitary common α mRNA level. These relations also fit the idea of the lower sensitivity of the FSH secretion to starvation, since pituitary FSHβ mRNA, which is considered to be an important factor influencing FSH secretion, responded to starvation weakly and slowly. Similar results were obtained by Kobayashi and Ishii (2002).

There were differences between experimental and control groups in the results of correlation analyses of gonadotropin levels and mRNA levels. The plasma LH level showed no significant correlation with the pituitary LHβ mRNA level in the control birds but a significant positive correlation was found in the experimental birds. This discrepancy can be explained by the emergence of individuals with extremely low plasma LH and pituitary LHβ mRNA levels in the experimental or starved birds. If we exclude individuals with such low values (less than 0.5 in the LHβ mRNA level and less than 1.0 ng/ml in plasma LH level) in the experimental birds, the positive correlation disappears. This may be explained by assuming that there is a deposit of the LH β-subunit in gonadotrophs that is sufficient to produce LH necessary for secretion in the normal condition. In the extreme condition such as severe starvation, the deposit of LH β-subunit would disappear and urgent synthesis of the LH β-subunit may be initiated.

Different responses to starvation between LH and FSH may be caused originally by different sensitivity of LH and FSH secretion to gonadotropin-releasing hormone (GnRH). Hattori *et al*. (1986) studied changes in plasma and pituitary LH and FSH levels in Japanese quail stimulated with GnRH and concluded that the secretion of LH is solely and rigidly controlled by GnRH but the secretion of FSH is at least partly autonomous. In mammals, Bergendahl and Veldhuis (1995) mentioned in their review that inadequate nutritional intake impaired LH secretion mainly via GnRH release from hypothalamus. Similarly, in the chicken, it was suggested that starvation suppressed release of GnRH from the median eminence (Contijoch *et al*., 1992). However, it was suggested in the chicken that pituitary sensitivity to GnRH declined in an early phase of starvation because administration of GnRH had no effect on the plasma LH level in that phase (Tanabe *et al*., 1981). Vanmontfort *et al*. (1994) reported in the hen that a decline in the plasma inhibin level was accompanied by an increase in the plasma FSH level while plasma LH and progesterone levels decreased in short-term food deprivation. Not only the suppression of GnRH release but also an additional unknown factor or factors must be taken into consideration as the cause of the difference in the response to starvation between LH and FSH.

It has been reported that starvation had a suppressive effect on levels of FSHβ and common α mRNAs but no effect on the level of LHβ mRNA in the pituitary gland in the rat (Bergendahl *et al*., 1989, 1991; Bergendahl and Huhtaniemi, 1993), and that long-term food restriction had no effect on levels of gonadotropin subunit mRNAs in the sheep (Thomas *et al*., 1990), despite plasma levels of gonadotropins being suppressed and pituitary contents unchanged or decreased by starvation in both animals (Bergendahl *et al*., 1989; Thomas *et al*., 1990; Bergendahl *et al*., 1991; Bergendahl and Huhtaniemi, 1993). In the Japanese quail, however, suppression of plasma gonadotropin levels was accompanied by suppression of pituitary gonadotropin subunit mRNA levels (Kobayashi and Ishii, 2002; present study). Our results in the Japanese quail seem to show that starvation affects pituitary gonadotropin subunit mRNA and plasma gonadotropin levels more severely in birds than in mammals. This reminds us that birds generally need continuous feeding.

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We also found in the present study that T4 did not show clear changes during the food deprivation and re-feeding periods but T3 showed a marked decrease during the food-deprivation period followed by a rapid recovery during the re-feeding period. We have reported similar changes in T3 and T4 levels during the food-deprivation period in the Japanese quail (Kobayashi and Ishii, 2002). Also, in the present study, plasma T4 levels showed significant negative correlations with pituitary TSHβ mRNA levels in both control and experimental groups. A hypothesis to explain the present result would be a negative feedback effect of thyroid hormone on TSH. Larsen (1982) mentioned in a review that the ratio of nuclear T3 derived from intracellular conversion of T4 in the anterior pituitary is larger than that in the liver or kidney in the rat. In other words, plasma T4 is more important than plasma T3 as a source of nuclear T3 in the pituitary gland. Some investigators (May, 1978; Klandorf and Harvey, 1985; Hoshino *et al*., 1988; Geris *et al*., 1999; Van der Geyten *et al*., 1999) reported that starvation decreased the plasma T3 level but increased the plasma T4 level in the chicken, while Geris *et al*. (1999) and Van der Geyten *et al*. (1999) reported that starvation decreased the plasma TSH level in the same animal. However, in mammals, it was reported that starvation decreased both plasma T4 and T3 levels (Burger *et al*., 1980; Chopra, 1980; Tveit and Almid, 1980; Gavin and Moeller, 1983; Tveit and Larsen, 1983; O'Mara *et al*., 1993), and also the plasma TSH level (Campbell *et al*., 1977; Tveit and Almid, 1980; Tveit and Larsen, 1983; Hugues *et al*., 1988). It was also reported in the rat that intra-pituitary conversion of T4 to T3 was not changed or was decreased slightly by starvation (Cheron *et al*., 1979; Kaplan, 1980) and also secretion of thyrotropin-releasing hormone (TRH) from the hypothalamus was decreased by starvation (Rondeel *et al*., 1992; van Haasteren *et al*., 1995). A reduction of the release of TRH by starvation was also suggested in the chicken (Geris *et al*., 1999).

It is clear in the present and previous studies on starvation that the plasma T3 level is drastically decreased by starvation. One of the main sites of the thyroid hormone metabolism is the liver. In the Japanese quail, it has been reported that starvation decreased hepatic T3 production from T4 (Hughes and McNabb, 1986). In the chicken, however, starvation increased hepatic 5-deiodination (degradation of T3 to 3,3'-diiodothyronine) while hepatic 5'-deiodination (production of T3 from T4) was not affected (Darras *et al*., 1995, 1998; Van der Geyten *et al*., 1999). In the rat, it was reported that starvation decreased hepatic 5'-deiodination (O'Mara *et al*., 1993; Darras *et al*., 1995) and increased hepatic 5-deiodination (Darras *et al*., 1995, 1998).

Little information has been available on changes in endocrine parameters during the recovery phase from starvation and no information has been available on gonadotropin subunit mRNA levels during the recovery phase. It is noteworthy in the present study that the TSHβ subunit mRNA level showed a statistically insignificant decrease during the food-deprivation period but a statistically significant transitory increase in the early re-feeding period. This pattern of the change in the TSHβ mRNA level coincided with that of the change in the hepatic weight as was pointed out already. We may expect a temporary rise of the TSH secretion at the peak of the TSH β mRNA level. Supporting our findings on the change in the TSHβ subunit mRNA level during the recovery phase, it was reported that the plasma TSH level exceeded the control level after the start of re-feeding in the rat (Hugues *et al*., 1983; Rondeel *et al*., 1992) and bull (Tveit and Larsen, 1983). However, van Haasteren *et al*. (1995) reported that temporary increases in plasma TSH and pituitary TSHβ mRNA levels by re-feeding were not observed in the rat. A similar result in plasma TSH level has been reported in the chicken (Van der Geyten *et al*., 1999). More detailed studies are needed to clarify the relationship between food availability and hypothalamic and peripheral mechanisms controlling thyroid function and thyroid hormone metabolism in birds.

The testicular weight and size of cloacal protrusion decreased during food-deprivation period. These results are harmonious with the fact that the plasma LH level was decreased by the starvation. It has been reported in the chicken (Tanabe *et al*., 1981; Hoshino *et al*., 1988) and rat (Howland, 1975; Xie, 1991) that suppressed secretion of LH by starvation could be recovered to the initial normal level within several days when the term of starvation had been finished and then animals were fully fed. In the present study, we confirmed their findings and furthermore added new information that levels of gonadotropin subunit mRNAs are recovered simultaneously within several days.

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