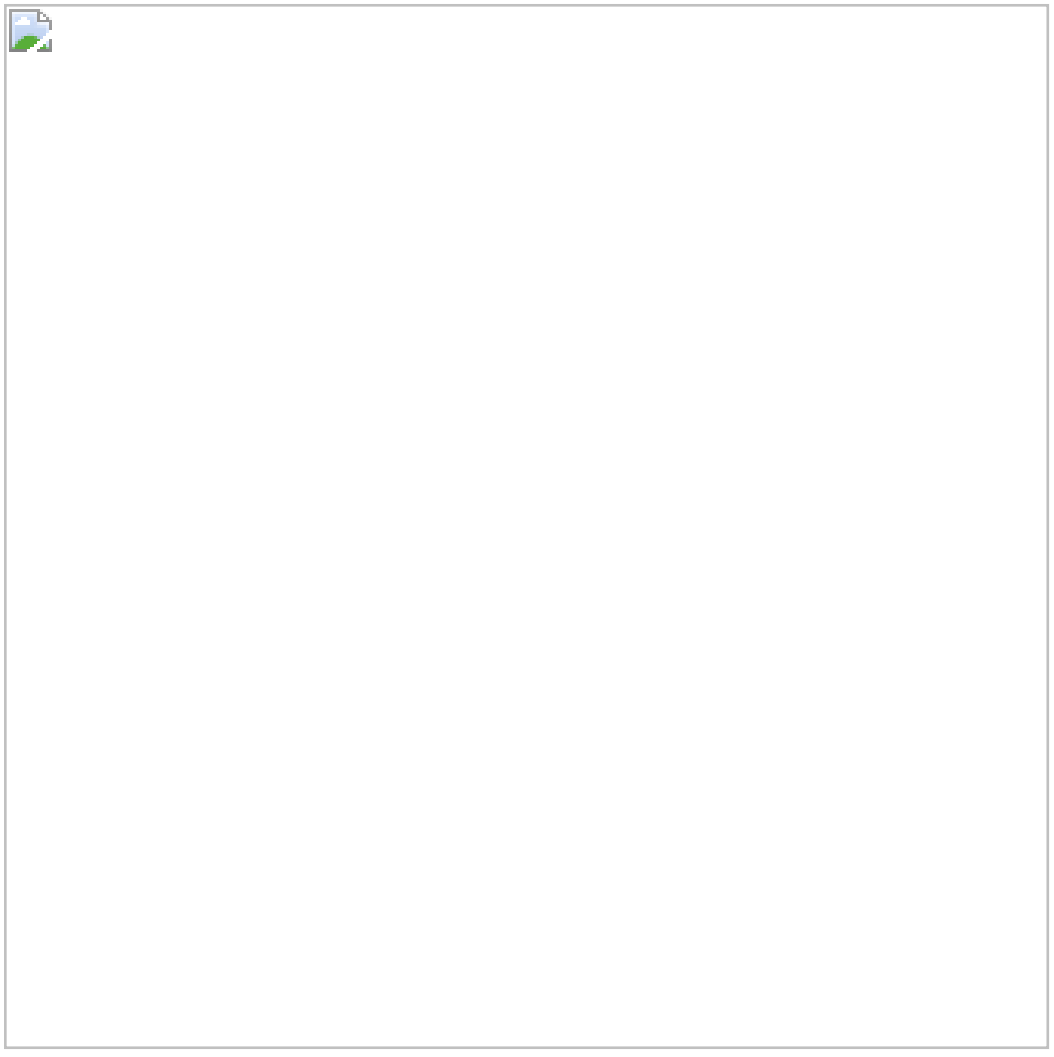

Effects of Starvation on Gonadotropin and Thyrotropin Subunit mRNA Levels and Plasma Hormone Levels in the Male Japanese Quail (Coturnix coturnix japonica)

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Effects of Starvation on Gonadotropin and Thyrotropin Subunit mRNA Levels and Plasma Hormone Levels in the Male Japanese Quail (*Coturnix coturnix japonica*)

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ABSTRACT—Contents of mRNAs encoding LHβ-, FSHβ-, TSHβ- and common a-subunit precursor molecules were measured in male Japanese quail deprived of food for three days. Plasma LH, FSH, thyroxine and triiodothyronine levels were also measured in the same birds. Plasma LH levels declined during the period of food deprivation. Levels in starved birds were not different from those in control birds after one day of starvation but were significantly lower after three days. Plasma FSH levels showed a similar decline, although the changes were not significant. Plasma thyroxine levels did not decrease during starvation, whilst plasma triiodothyronine levels decreased drastically and significantly soon after the start of starvation. All the hormone subunit mRNA contents in starved birds also decreased, with differences from control birds significant 3 days after the start of starvation. Plasma FSH levels showed a strong positive correlation with pituitary FSHβ mRNA levels, while plasma LH levels had a strong positive correlation with common α mRNA levels and practically no correlation or even a negative correlation with LHβ mRNA levels. These results suggest that starvation suppresses not only gonadotropin and thyrotropin secretion but also their synthesis in the pituitary gland. Furthermore, these results showed that FSH and LH have different synthesis and secretion dynamics in the Japanese quail. Contradicting results with TSHβ mRNA and thyroid hormones lead us to assume that starvation affects thyroid hormone metabolism in peripheral tissue, presumably in the liver.

Key words: starvation, gonadotropin, thyrotropin, mRNA, Japanese quail

INTRODUCTION

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 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; Scanes *et al*., 1976; Campbell *et al*., 1977; Tanabe *et al*., 1981; Badger *et al*., 1985; Hoshino *et al*., 1988; Foster *et al*., 1989). Starvation can be caused accidentally by husbandry problems in domestic and experimental birds, and by changes in the natural environmental condition such as the storm, flood, and snow in feral birds. The relation between food availability and gonadotropic endocrine activity is thus an important topic in both veterinary endocrinology and ecological endocrinology. However, it has not been known whether starvation affects reproductive activity by stopping only the secretion of the hormones or by suppressing both secretion and synthesis of the hormones.

The measurement of intra-pituitary contents of mRNAs encoding the gonadotropin subunit precursor molecules is a useful approach to this problem. A series of papers with such approach has been published for the rat (Bergendahl *et al*., 1989 and 1991; Bergendahl and Huhtaniemi, 1993), with a similar paper also on the sheep (Thomas *et al*., 1990).

Birds are known as animals that need continuous feeding, and hence they might be extremely sensitive to starvation. However, there has been no published study on the change in the gonadotropin subunit mRNA content in starved birds. Accordingly, we studied changes in pituitary contents of mRNAs encoding the subunit precursor molecules of gonadotropins and thyrotropin in food deprived male Japanese quail.

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MATERIALS AND METHODS

Animal and treatments

Adult male Japanese quail (10-weeks old) were purchased from a commercial dealer (Tokai Yuki Co., Aichi Prefecture, Japan). The quail were kept separately in individual cages under constant conditions of illumination (16h light: 8h darkness) and temperature (20°C) for 2 weeks before the experiment started. They were provided with food (NQ-1, Nisseiken, Tokyo, Japan) and water *ad libitum*.

Thirty seven quail were randomly divided into 3 control and 2 experimental groups. Quail in the two experimental groups were deprived of food but had continuous access to water. Birds in one of the experimental groups were sacrificed one day after the onset of food deprivation, and birds of the other experimental group were sacrificed 3 days after the onset of the food deprivation. Birds of the 3 intact control groups were also sacrificed just before the onset of food deprivation, one day after and 3 days after the food deprivation in the experimental groups, respectively. Each group consisted of 7 or 8 birds.

The 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 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 by 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 (pQF6111) 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 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

Total RNA samples were denatured in 6.5% formaldehyde, 50% deionized formamide, 0.5xMOPS (1xMOPS: 0.02M 3-morpholinopropanesulfonic acid, 0.05M sodium acetate trihydrate, 1mM EDTA) at 65°C for 10 min, and then rapidly cooled on ice. Denatured total RNA samples were applied to a plate of agarose-formaldehyde gel (1.2% agarose and 8% formaldehyde in 1xMOPS) and then electrophoresis was performed in 8% formaldehyde 1xMOPS buffer. After electrophoresis, separated samples were transferred to a nylon membrane (NYTRAN, Schleicher & Schell, Postfach, Germany). As the reference standard, 6.5 μg of total RNA extracted from pituitaries pooled from 65 adult male Japanese quail was applied to every plate and electrophoresed. Prehybridization was performed at 42°C for 3hr in hybridization buffer [6xSSC (1xSSC: 150mM sodium citrate, 15mM sodium chloride, pH 7.0), 0.1% sodium dodecylsulfate (SDS), Denhardt's solution (0.02% BSA fraction V, 0.02% ficoll type 400 and 0.02% poryvinylpyrolidone)] containing salmon sperm DNA (0.2mg/ml) and then hybridization was performed in the hybridization buffer containing labeled probe at 60°C overnight. Specific radioactivity of the labeled probe was ca. 40 MBq/μg. Membranes were then soaked in 3xSSC, 0.1% SDS at 60°C and washed 3 times in 1xSSC, 0.1% SDS at 60°C for 20 min. Hybridization signals on the membranes were analyzed and quantified in the BAS-2000 II Bio-Imaging analyzer (Fuji Photo Film Co., Ltd., Japan). After signal quantification, membranes were boiled twice in 0.1xSSC, 1%SDS for 15min for removing labeled probe on membranes and then re-used for hybridizations with the other probes. Hybridization signals for all the mRNAs were expressed as relative values to the signal of the reference standard in each electrophoretic run and 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 or total RNA contents 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

Student's t-test or Mann-Whitney U test was applied for comparison of means between two groups on the same day. One-way analysis of variance or Kruskal-Wallis test was applied for comparison of means among the each group. Relationships between plasma hormone levels and pituitary hormone subunit mRNA levels were analyzed by simple, partial and multiple correlations. A P value less than 0.05 was chosen to be statistically significant.

RESULTS

Body weight, testicular weight and cloacal protrusion area

Both the mean body weight and mean cloacal protrusion area in the starved group showed time dependant decreases, while these parameters stayed almost constant during the same period in the control group (Fig. 1A and B). The mean testicular weight showed a slight decrease with time in the starved group but a slight increase in control group, although these changes were statistically insignificant (Fig. 1C). The mean body weight in the one-day starved group was significantly lower than that in its control group ($P<0.05$; Fig.1A). The mean cloacal protrusion area and mean testicular weight in the one-day starved group were not significantly different from corresponding mean values in the control group (Fig. 1B and C). Values of all the three parameters in the 3 days starved group were significantly different from the values in the 3 days control group ($P<0.01$; Fig. 1A, B and C). When testicular weights were expressed as the ratio to body weights, there were no significant differences in the values between the starved and control groups on either day (Fig. 1D).

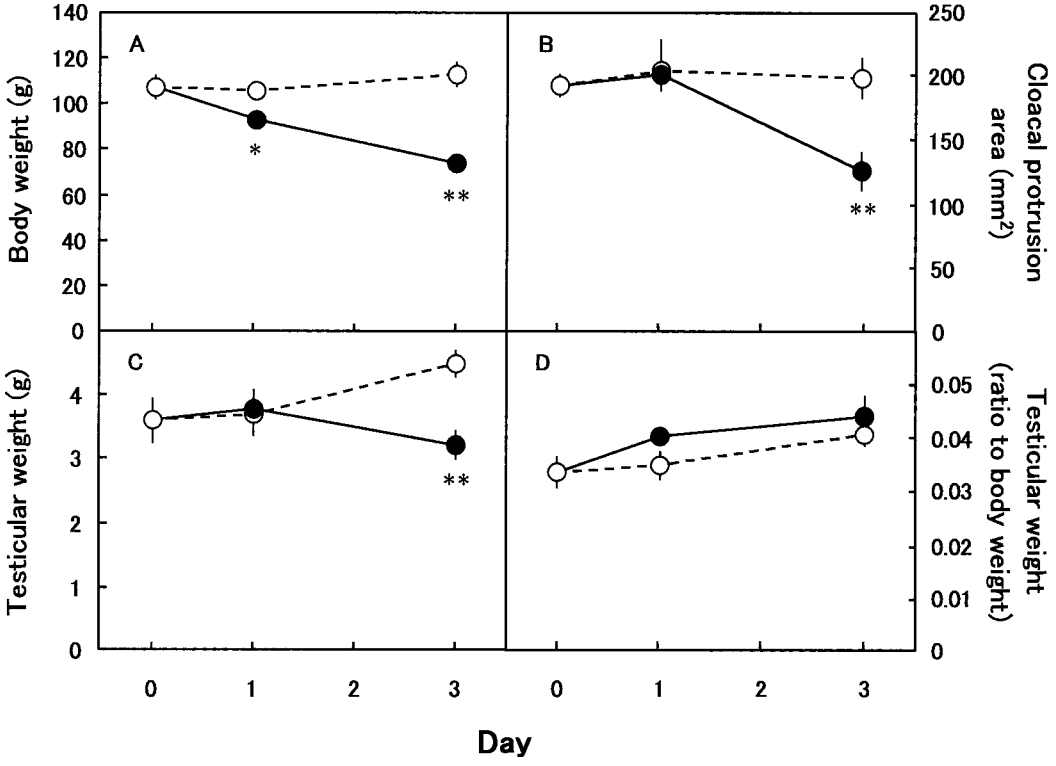

Fig. 1. Changes in body weight (A), cloacal protrusion area (B), testicular weight (C) and testicular weight expressed as the ratio to body weight (D) in starved (solid circles with solid line) and control (open circle with broken line) quail. Circles and bars represent the mean±SEM. Significant differences between the starved and control groups on the same day are indicated at P<0.05 (*) and P<0.01 (**).

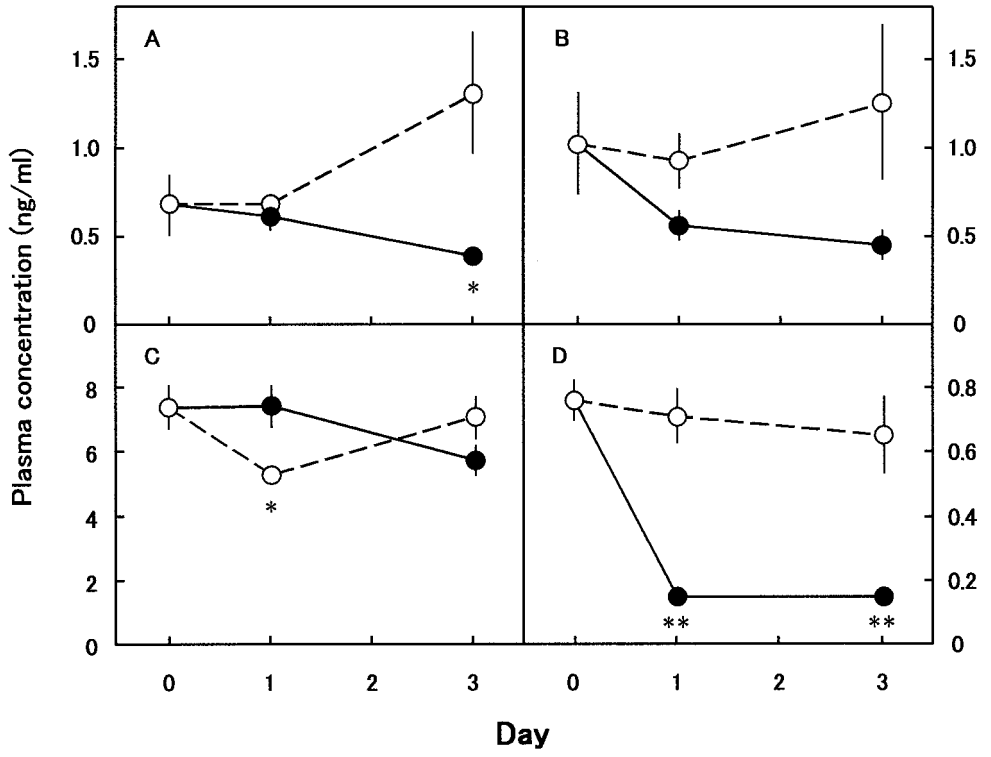

Fig. 2. Changes in plasma concentrations of luteinizing hormone (LH; A), follicle-stimulating hormone (FSH; B), thyroxine (T4; C) and triiodothyronine (T3; D) in starved (solid circles with solid line) and control (open circle with broken line) quail. Circles and bars represent the mean±SEM. Significant differences between the starved and control groups on the same day are indicated at P<0.05 (*) and P<0.01 (**).

Plasma concentrations of gonadotropins and thyroid hormones

Mean plasma LH levels in the starved groups decreased steadily with time, while levels did not change in the one day control group and increased insignificantly in the 3 day control group (Fig. 2A). The difference in levels between the starved and control groups was significant 3 days after the start of starvation ($P<0.05$). Mean plasma FSH concentrations in both the starved and control groups showed similar changes to mean plasma LH concentrations (Fig. 2B). However, there were no significant differences between the starved and control groups on either day. The mean plasma T4 concentration in the control group showed a slight decrease a day after and then a slight increase 3 days after the start of starvation, although these changes were statistically insignificant. Mean plasma T4 concentrations in the starved group showed almost opposite changes, with no increase one day after and then a statistically insignificant decrease 3 days after the start of starvation (Fig. 2C). The difference between the starved and control groups was significant a day after ($P<0.05$) but not significant 3 days after the start of starvation. Mean plasma T3 concentrations in the starved group decreased abruptly one day after the start of starvation (Fig. 2D) and were below the limit of detection (0.15 ng/ml) in all of birds in both one day and 3 day starved groups. The concentrations in the control group showed a slight but statistically insignificant decrease with time. The differences between starved and control groups were significant both one day and 3 days after the start of starvation ($P<0.01$).

LHβ, FSHβ, TSHβ and common α mRNA pituitary contents

Pituitary contents of LHβ, FSHβ, TSHβ and common α mRNA were expressed in two different ways: one in terms of the ratio of each hormone subunit mRNA content to the β-actin mRNA content (Fig. 3), and the other the ratio of each hormone subunit mRNA content to the total RNA content (Fig. 4).

When contents of the hormone subunit mRNAs were expressed as the ratio to β-actin mRNA, all the hormone β-subunit mRNA levels showed similar changes with time (Fig. 3A, B and C). The control group did not show clear change with time, but the starved group decreased unanimously with time down to about 50% or less than of the control level 3 days after the starvation was started. Differences in all the β-subunit mRNA levels between the starved and control levels were not significant 1 day after but significant 3 days after the start of starvation ($p<0.05$). The common α mRNA level also showed a similar change, but the mean level of the starved group decreased to a greater extent to 22% of the mean control level 3 days after the start of starvation (Fig. 3D). The difference between the starved and control levels was highly significant ($P<0.01$).

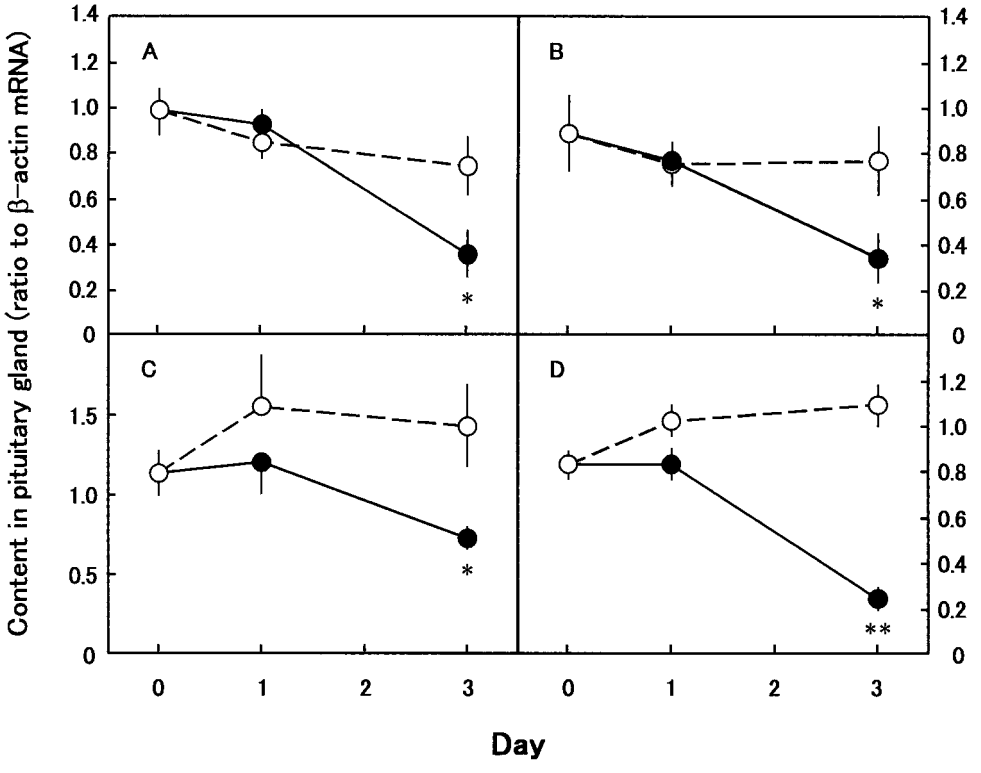

Fig. 3. Changes in pituitary contents of LHβ (A), FSHβ (B), TSHβ (C) and common α (D) mRNA expressed as the ratio to β-actin mRNA content in starved (solid circles with solid line) and control (open circle with broken line) quail. Circles and bars represent the mean±SEM. Significant differences between the starved and control groups on the same day are indicated at $P<0.05$ (*) and $P<0.01$ (**).

When hormone subunit mRNA levels were expressed as the ratio to the total RNA content, they showed less marked changes, and LHβ, FSHβ and TSHβ mRNA levels showed no significant difference from control levels at any time point (Fig. 4A, B and C). However, the common α mRNA level showed a significant difference from the control level 3 days after the start of starvation ($P<0.01$; Fig. 4D).

Mean β-actin mRNA contents fluctuated within small and insignificant ranges, and no significant difference was observed between the starved and control groups both one day and 3 days after the start of starvation (Fig. 5). Total RNA content varied in a wider range than the β-actin mRNA

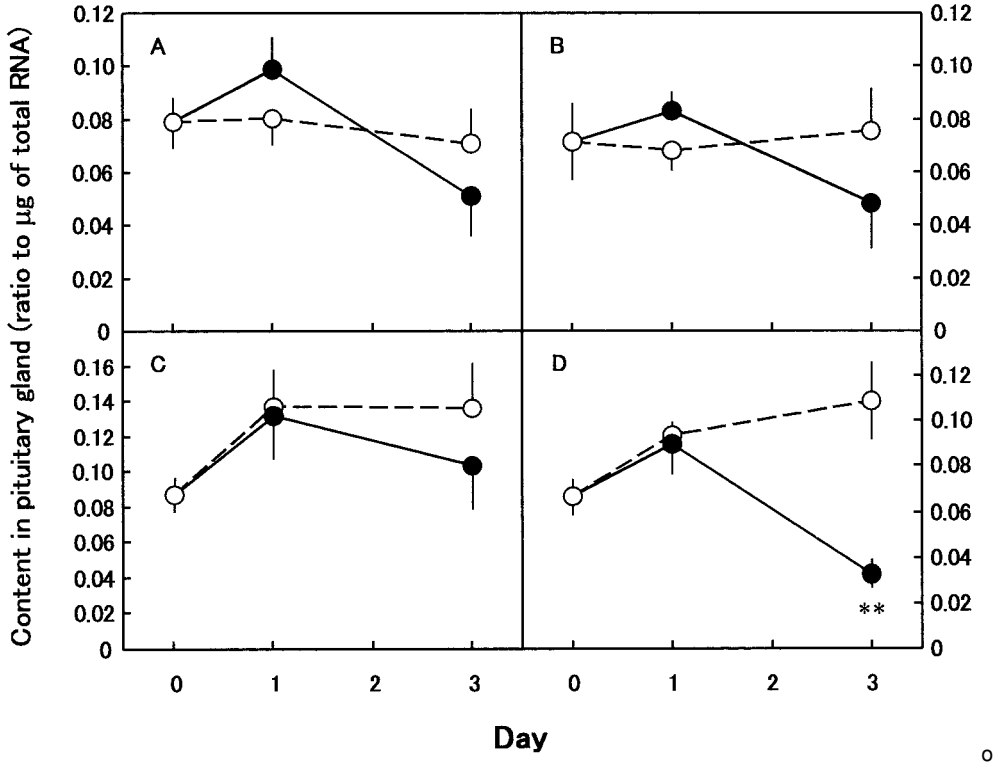

Fig. 4. Changes in pituitary contents of the LHβ (A), FSHβ (B), TSHβ (C) and common α (D) mRNA expressed as the ratio to total RNA content in starved (solid circles with solid line) and control (open circle with broken line) quail. Circles and bars represent the mean± SEM. Significant differences between the starved and control groups on the same day are indicated at $P<0.01$ (**).

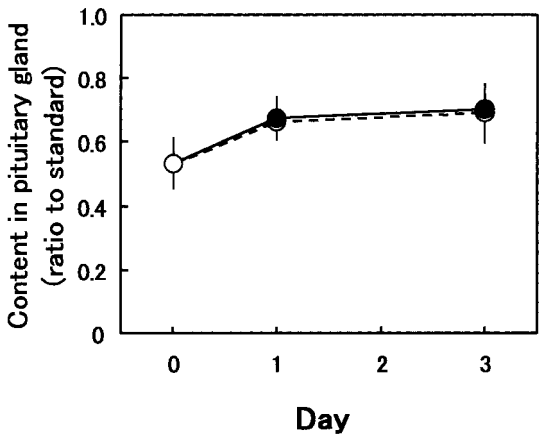

Fig. 5. Changes in pituitary contents of the β-actin mRNA in starved (solid circles with solid line) and control (open circle with broken line) quail. The contents are expressed as the ratio to a standard quail pituitary RNA preparation (see Materials and Methods). Circles and bars represent the mean±SEM.

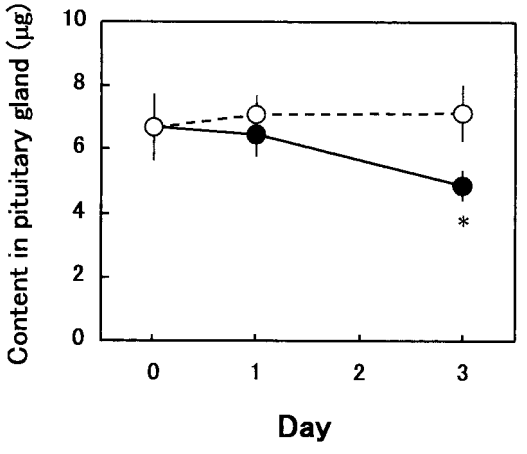

Fig. 6. Changes in pituitary contents of total RNA in starved (solid circles with solid line) and control (open circle with broken line) quail. Circles and bars represent the mean±SEM. Significant differences between the starved and control groups on the same day are indicated at $P<0.05$ (*).

content from one day after to 3 days after the starvation was started in the starved group (Fig. 6). The difference between the starved and control levels was significant ($P<0.05$).

Correlation analyses

For correlation analyses on hormone subunit mRNA levels, the ratio of each mRNA content to the β-actin mRNA content was used. We expected that individuals with higher hormone levels would contain higher mRNA levels of the hormone subunits, so we analyzed relations between plasma hormone level and hormone subunit mRNA level by simple and partial correlations. Scatter diagrams of plasma hormone levels and hormone subunit mRNA levels are shown in Fig. 7, 8, 9 and 10, and correlation coefficients in simple and partial correlation analyses are shown in Table 1 and 2.

Simple correlation

Plasma LH levels showed practically no correlation with LHβ mRNA levels, but a high and significant positive corre-

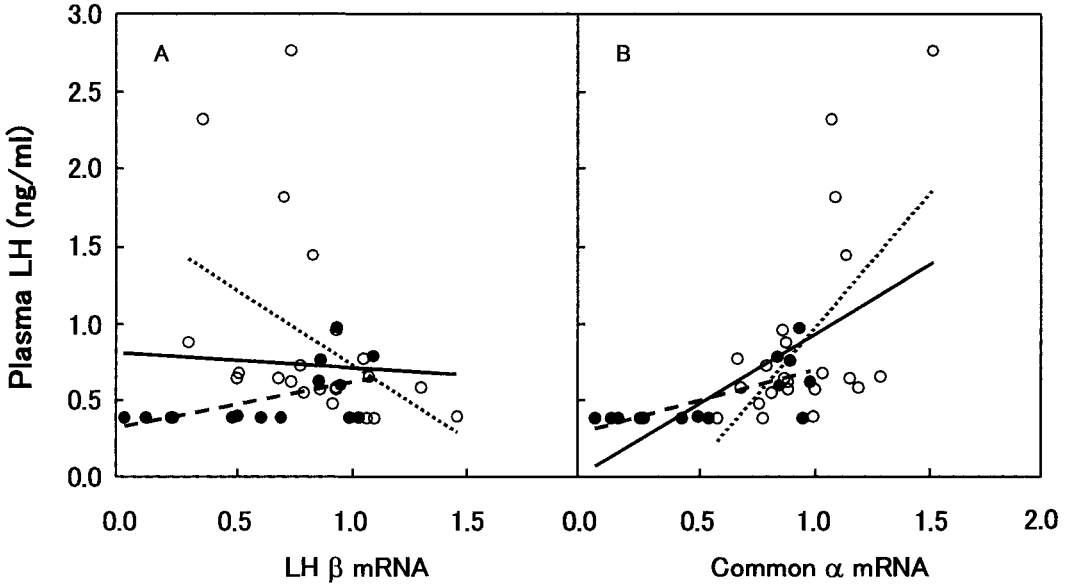

Fig. 7. Correlations between plasma concentrations of LH and pituitary contents of LHβ mRNA (A) and common α mRNA (B). Solid circles, starved group; open circle, control group. Regression lines are shown as dotted line (control group), broken line (starved group) and solid line (combined).

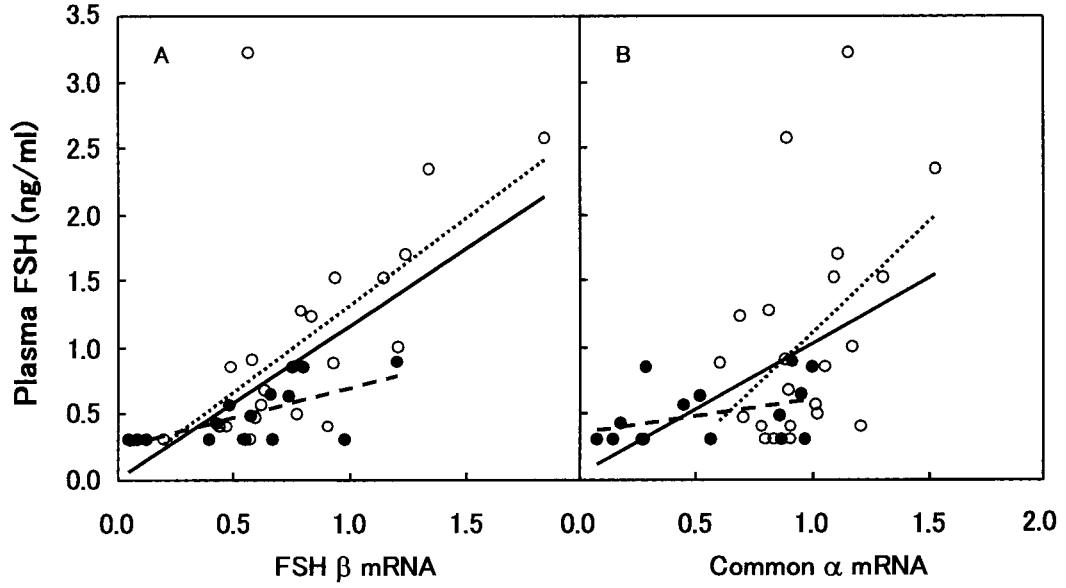

Fig. 8. Correlations between plasma concentrations of FSH and pituitary contents of FSHβ mRNA (A) and common α mRNA (B). Solid circles, starved group; open circle, control group. Regression lines are shown as dotted line (control group), broken line (starved group) and solid line (combined).

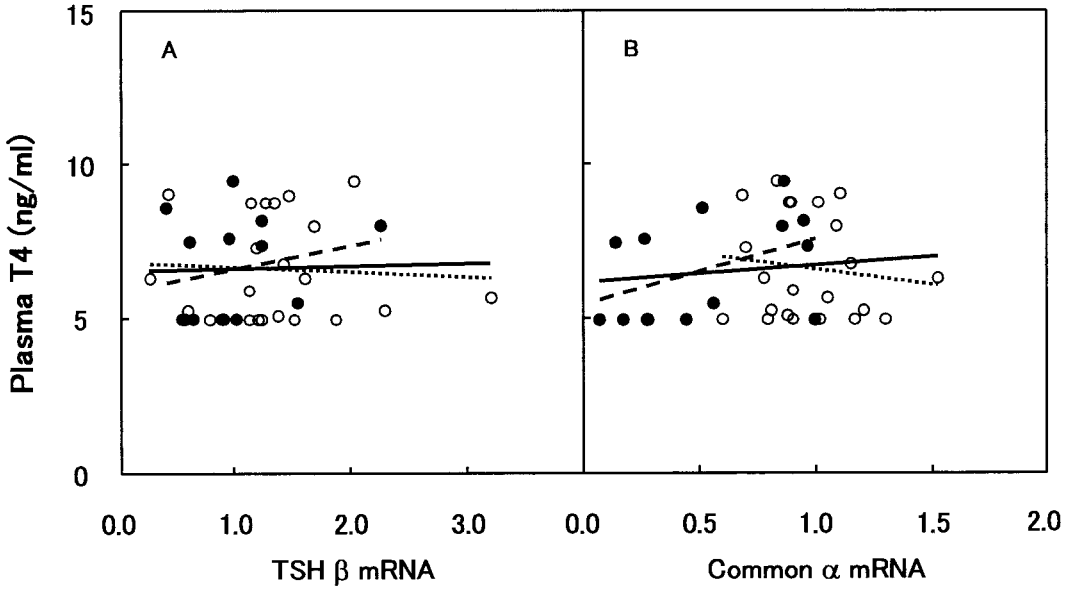

Fig. 9. Correlations between plasma concentrations of T4 and pituitary contents of TSHβ mRNA (A) and common α mRNA (B). Solid circles, starved group; open circle, control group. Regression lines are shown as dotted line (control group), broken line (starved group) and solid line (combined).

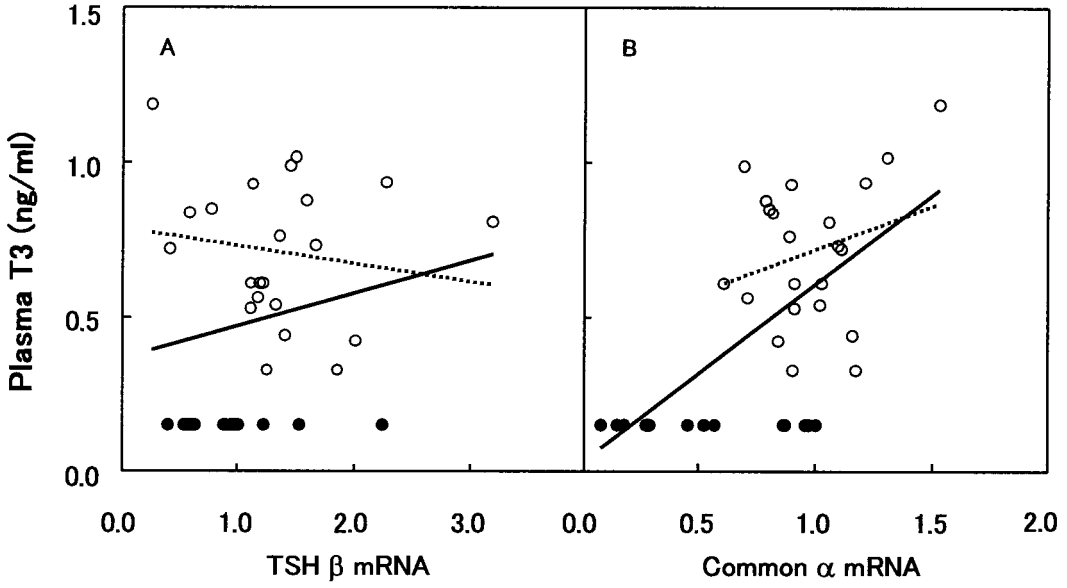

Fig. 10. Correlations between plasma concentrations of T3 and pituitary contents of TSHβ mRNA (A) and common α mRNA (B). Solid circles, starved group; open circle, control group. Regression lines are shown as dotted line (control group), broken line (starved group) and solid line (combined).

lation with common α mRNA levels (Fig. 7 and Table 1). Plasma FSH levels showed significant positive correlations with both FSHβ mRNA and common α mRNA levels (Fig. 8 and Table 1). Plasma T4 levels showed no significant correlation with either TSHβ mRNA or common α mRNA levels (Fig. 9 and Table 1). Plasma T3 levels showed a weak insignificant positive correlation with TSHβ mRNA levels, but a significant positive correlation with common α mRNA levels (Fig. 10 and Table 1).

To determine whether the relation between pituitary mRNA and plasma hormone levels was different between control and starved group, correlation analyses were performed for control and starved groups separately. When simple correlation coefficients were calculated only in the control group, plasma LH levels showed a significantly negative correlation with LHβ mRNA levels and a positive correlation with common α mRNA levels (Fig. 7 and Table 2). Plasma FSH levels showed significantly positive correlations

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

Dependent variable	Independent variable	r in simple correlation (P)	r in partial correlation (P)	Fixed variable in partial correlation
LH	LHβ	-0.0595 (0.7266)	-0.5320 (0.0008)***	common α
LH	common α	0.5833 (0.0002)**	0.7248 (<0.0001)***	LHβ
FSH	FSHβ	0.6176 (<0.0001)***	0.4676 (0.004)**	common α
FSH	common α	0.4908 (0.002)**	0.2027 (0.2359)	FSHβ
T4	TSHβ	0.0307 (0.8589)	-0.0160 (0.9275)	common α
T4	common α	0.1208 (0.4829)	0.1179 (0.4998)	TSHβ
T3	TSHβ	0.1935 (0.2580)	-0.0436 (0.8038)	common α
T3	common α	0.6009 (0.0003)**	0.5810 (0.0003)**	TSHβ

Note. Plasma hormone levels were taken as independent 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<0.01 (**) and P<0.001 (***).

Table 2. Simple and partial correlation analyses between plasma hormone levels and its corresponding hormone subunit mRNA level for the control and starved groups.

Dependent variable	Independent variable	r in simple correlation (P)	r in partial correlation (P)	Fixed variable in partial correlation
Control group				
LH	LHβ	-0.4301 (0.0457)*	-0.4670 (0.0328)*	common α
LH	common α	0.6071 (0.0027)**	0.6279 (0.0023)**	LHβ
FSH	FSHβ	0.6040 (0.0029)**	0.5253 (0.0145)*	common α
FSH	common α	0.4497 (0.0358)*	0.3011 (0.1847)	FSHβ
T4	TSHβ	-0.0630 (0.7805)	-0.0620 (0.7894)	common α
T4	common α	-0.1306 (0.5623)	-0.1302 (0.5739)	TSHβ
T3	TSHβ	-0.1562 (0.4875)	-0.1652 (0.4743)	common α
T3	common α	0.2636 (0.2388)	0.2688 (0.2388)	TSHβ
Starved group				
LH	LHβ	0.5678 (0.0272)*	-0.1242 (0.6724)	common α
LH	common α	0.7103 (0.0030)**	0.5292 (0.0517)	LHβ
FSH	FSHβ	0.6385 (0.0104)*	0.5687 (0.0338)*	common α
FSH	common α	0.3797 (0.1628)	-0.1494 (0.6102)	FSHβ
T4	TSHβ	0.2261 (0.4370)	-0.0536 (0.8620)	common α
T4	common α	0.4461 (0.1099)	0.3978 (0.1872)	TSHβ

Note. Plasma hormone levels were taken as independent 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 (*) and P<0.01 (**).

with both FSHβ mRNA and common α mRNA levels (Fig. 8 and Table 2), and plasma T4 and T3 levels showed no significant correlation with either TSHβ mRNA or common α mRNA levels (Fig. 9 and 10, and Table 2). In the starved group, plasma LH levels showed significantly positive correlations with both LHβ and common α mRNA levels (Fig. 7 and Table 2). Plasma FSH levels showed a significantly positive correlation with FSHβ mRNA levels, but an insignificant correlation with common α mRNA levels (Fig. 8 and Table 2), and plasma T4 levels showed no correlation with TSHβ or common α mRNA levels (Fig. 9 and Table 2). Plasma T3 levels in the starved group were all below the minimal detectable level and we did not apply the simple correlation analysis to them.

Partial correlation

To exclude the effect of one of two independent factors that could influence the plasma hormone level, we performed partial correlation analyses in which effect of one of the factors was fixed. These analyses give a clearer relation between the non-fixed independent factor and dependent factor than do simple correlation analyses.

When partial correlation coefficients were calculated for the combination of the control and starved groups, plasma LH levels showed a significantly negative correlation with LHβ mRNA levels but a positive significantly correlation with common α mRNA levels (Table 1). Plasma FSH levels showed a significantly positive correlation with FSHβ mRNA levels, but no correlation with common α mRNA levels (Table 1). Plasma T4 levels showed no correlation with

either TSHβ mRNA or common α mRNA levels (Table 1). Plasma T3 levels showed no correlation with TSHβ mRNA levels, but a significantly positive correlation with common α mRNA levels (Table 1).

In addition, we performed partial correlation analyses for control and starved groups separately. When partial correlation coefficients were calculated only in the control group, results were similar to those obtained in the simple correlation except that plasma LH levels were more strongly positively correlated with common α mRNA levels and negatively correlated with LHβ mRNA levels, whereas plasma FSH levels were more weakly positively correlated with both FSHβ and common α mRNA levels (Table 2). In the starved group, all of the plasma hormone levels were less strongly correlated with the pertinent pituitary hormone mRNA levels compared with the results in the simple correlation and a significant correlation was observed only between plasma FSH and FSHβ mRNA levels (Table 2).

Multiple correlation

To analyze the combined relationships between hormone-specific β-subunit mRNA and common α mRNA levels and plasma hormone levels, we calculated multiple correlation coefficients taking two subunit mRNA levels which were presumed to be pertinent as independent variables and a plasma hormone level as the dependent variable. Correlation coefficients in multiple correlation analyses are shown in Table 3.

When multiple correlation coefficients were calculated for the combination of the control and starved groups, for the control group alone and for the starved group alone, plasma LH and FSH levels showed significant correlations with β-subunit mRNA for each hormone and common α mRNA levels. Plasma T4 levels were not correlated with TSHβ mRNA and common α mRNA levels, but plasma T3 levels were correlated with TSHβ mRNA and common α mRNA levels.

Table 3. Multiple correlation analysis between plasma hormone levels and its corresponding hormone subunit mRNA levels for the combination of the control and starved groups, for the control group alone and for the starved group alone

Dependent variable	Independent variables	r in multiple correlation (P)
Combination of both the control and starved groups		
LH	LHβ and common α	0.7260 (<0.002)**
FSH	FSHβ and common α	0.6378 (<0.002)**
T4	TSHβ and common α	0.1218 (0.7817)
T3	TSHβ and common α	0.6019 (0.0020)**
Control group		
LH	LHβ and common α	0.7115 (<0.002)**
FSH	FSHβ and common α	0.6499 (0.0059)**
T4	TSHβ and common α	0.1444 (0.8187)
T3	TSHβ and common α	0.2941 (0.4230)
Starved group		
LH	LHβ and common α	0.7156 (0.0137)*
FSH	FSHβ and common α	0.6488 (0.0371)*
T4	TSHβ and common α	0.4487 (0.2905)

Note. Plasma hormone levels were taken as independent 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 (*) and P<0.01 (**).

DISCUSSION

In the present study with the Japanese quail we confirmed previous findings that starvation inhibits the secretion of gonadotropins in mammals and birds (Negro-Vilar *et al*., 1971; Root and Russ, 1972; Howland and Skinner, 1973; Stewart *et al*., 1973; Campbell *et al*., 1977; Badger *et al*., 1985; Foster *et al*., 1989 in mammals and Scanes *et al*., 1976; Tanabe *et al*., 1981; Hoshino *et al*., 1988 in birds). We also showed that starvation induced decreases in mRNAs encoding the LHβ-, FSHβ- and common α-subunit precursor molecules in the Japanese quail. In the rat, it was reported that starvation had a suppressive effect on the levels of FSHβ and common α mRNAs and no effect on the level of LHβ mRNA in the pituitary gland (Bergendahl *et al*., 1989 and 1991; Bergendahl and Huhtaniemi, 1993). In the sheep, it was reported that long-term food restriction had no effect on the levels of gonadotropin subunit mRNA while it decreased levels of plasma gonadotropins (Thomas *et al*., 1990). In contrast to mammals, the present results show that starvation not only suppresses the secretion of gonadotropins but also decreases the content of mRNAs for the all gonadotropin subunits in the Japanese quail. The latter result suggests that the synthesis of gonadotropin is suppressed by starvation.

The present study also strongly suggests that the synthesis and secretion dynamics are different between FSH and LH. We found that plasma FSH levels were strongly positively correlated with pituitary FSHβ mRNA levels, whilst plasma LH levels were strongly positively correlated with common α mRNA levels but not with LHβ mRNA levels. These relations were consistently observed through the simple and partial correlations for the control group alone, the starved group alone and the combination of both groups, although the correlation between plasma LH levels and pituitary common α mRNA levels was marginally insignificant (r=0.5292, P=0.0517) in the partial correlation for the starved group.

Plasma LH levels showed practically no correlation with pituitary LHβ mRNA levels in the simple correlation for the combination of both the control and starved groups. However, a significant negative correlation was observed in the partial correlation for the combination of the control and starved groups and in the both simple and partial correlations for the control group alone, whereas a significantly positive correlation was observed in the simple correlation analysis and no significant correlation in the partial correlation analysis for the starved group alone. We can say that plasma LH levels were negatively correlated with pituitary LHβ mRNA levels, but that starvation decreased pituitary

LHβ mRNA to presumably nullify the negative correlation that had existed in the normal condition.

Plasma FSH levels were always positively and significantly correlated with FSHβ mRNA levels in the simple and partial correlations for the combination of both control and starved groups, for the control group alone and for the starved group alone. Plasma FSH levels were significantly correlated with common α mRNA levels only in the simple correlation for the combination of control and starved birds and for the control group alone. Plasma FSH levels were less strongly correlated with common α mRNA levels than with FSHβ mRNA levels. This fact suggests that plasma FSH levels were directly dependent on pituitary FSHβ mRNA levels, and the association of plasma FSH levels with pituitary common a mRNA levels occasionally observed would be less direct or important.

Hattori *et al*. (1986) studied changes in plasma and pituitary FSH and LH levels in Japanese quail stimulated with gonadotropin-releasing hormone (GnRH) and concluded that the secretion of LH is solely controlled by GnRH but the secretion of FSH is at least partly autonomous. They also suggested that the pituitary gland of the Japanese quail has a relatively large store of LH but little store of FSH. The lack of a positive correlation of plasma LH levels with pituitary LHβ mRNA levels and a strong and persistent positive correlation of plasma FSH levels with pituitary FSHβ mRNA levels we found in the present study are consistent with the difference in the hormone stores suggested by Hattori *et al*. (1986).

The last important finding in the present study is the effect of starvation on plasma T3 levels. As shown in Figs. 2 and 3, starvation decreased plasma T3 levels to about 21% of normal within 24 hr and also decreased pituitary TSHβ mRNA levels to 77% of the normal control level. The decease in pituitary TSHβ mRNA levels suggests a decrease in TSH secretion and hence T4 secretion. However, unexpectedly, we found that the starvation did not change plasma T4 levels significantly and also there were found no significant correlations between plasma T4 levels and pituitary TSHβ mRNA levels in any of the correlation analyses. Strangely, plasma T3 levels significantly and positively correlated with pituitary common α mRNA levels. However, the correlation between these two parameters disappeared in both the simple and partial correlations for the control group alone. Accordingly, we concluded that the significant positive correlation observed between plasma T3 levels and pituitary common α mRNA levels in the correlation analyses for the combination of the control and starved birds does not show a direct relation between these two parameters. Starvation caused both the decrease in the common α mRNA level and the decrease in the plasma T3 level independently and presumably through different physiological mechanisms.

Starvation has been reported to inhibit secretion of TSH in the rat (Campbell *et al*., 1977; Hugues *et al*., 1988), and the chicken (Geris *et al*., 1999; Van der Geyten *et al*., 1999). We found that the starvation decreased mRNA for the TSHβ precursor molecule in the Japanese quail. Similar results have been reported in the rat (Blake *et al*., 1991; Rodríguez *et al*. 1995). In the rat, Burger *et al*. (1980), Chopla (1980), Gavin and Moeller (1983) and O'Mara *et al*. (1993) reported that starvation decreased the plasma T4 and T3 levels. However, in the chicken, it has been reported that starvation decreased plasma T3 levels but increased plasma T4 levels (May, 1978; Klandorf and Harvey, 1985; Geris *et al*., 1999; Van der Geyten *et al*., 1999), while Geris *et al*. (1999) and Van der Geyten *et al*. (1999) found that starvation decreased plasma TSH levels. In the Japanese quail, we found that starvation rapidly decreased plasma T3 but not T4 levels. These results lead us to assume that starvation affects not only thyroid hormones secretions from the thyroid gland, but also thyroid hormone metabolism in peripheral tissues in Japanese quail. As is well known, T3 is formed from T4 by deiodination in the liver, kidney, pituitary gland and other tissues and hepatic function is much influenced by starvation. In the Japanese quail, it has been reported that starvation decreased hepatic T3 production from T4 (Hughes and McNabb, 1986). In the chicken, however, it was reported that 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 and 1998; Van der Geyten *et al*., 1999).

As mentioned above, we showed that starvation decreased the content of the TSHβ mRNA in the pituitary gland. This result suggests that TSH synthesis is suppressed by starvation in the Japanese quail. Further studies on the effects of starvation on the pituitary-thyroid axis and hepatic iodothyronine deiodination are needed to explain the relationship between TSH synthesis in pituitary gland and plasma thyroid hormone levels.

In the present study, we expressed quantities of hormone subunit mRNA in the pituitary gland in two different ways: in terms of the ratio to the β-actin mRNA content and to the total RNA content. Changes in the mRNA were less clear in the latter ratio. This is considered to be due to the fact that the total RNA content was affected by starvation but the β-actin mRNA content was not. Expression in terms of the ratio to the total RNA content should be avoided in starvation studies. The large decrease in the amount of total RNA induced by starvation may indicate that all the mRNAs were decreased in the pituitary gland. However this is not true because mRNA occupies a small portion of the total RNA, and the β-actin mRNA content did not change over three days of the starvation. Starvation may cause a decrease not only in specific mRNAs but also in other RNAs such as ribosomal RNA that are abundant in cells.

Starvation decreased the testicular weight and size of cloacal protrusion in the Japanese quail. The size of the cloacal protrusion reflects plasma androgen level in birds. Therefore, the significant decrease in the size of cloacal protrusion observed in the present study suggests that starva-

tion decreased plasma androgen levels.

We have shown that food deprivation for only one to three days can have serious effects on reproductive endocrine function in the male Japanese quail that has a relatively large body size. This suggests that feral birds with a smaller body size would be more seriously affected in their reproductive activities by unavailability of food even for a short period. In addition, when we use birds as experimental subjects, we should pay close attention to continuous feeding.

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