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The Effects of Fasting and Increased Feeding on Plasma Thyroid Hormones, Glucose, and Total Protein in Sea Turtles

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ABSTRACT—The effects of fasting and increased feeding on plasma thyroid hormones, glucose, and total protein levels were investigated in immature greens (*Chelonia mydas*) and Kemp's ridley (*Lepidochelys kempi*) sea turtles. Under constant temperature, nutritional status affected plasma thyroid hormones and glucose level, but not protein level in green and Kemp's ridley turtles. Plasma T_4 in green turtles decreased with deprivation of food, but did not do so in Kemp's ridleys. Increased feeding did not affect circulating levels of plasma T_4 in either species. Plasma T_3, in contrast, tended to decrease during fasting and increase with refeeding or increased feeding. Blood glucose level fell with deprivation of food, but remained constant during increased feeding. Sea turtles may use glycogen or lipid during short-term fasting as in endothermic vertebrates.

INTRODUCTION

In various vertebrates, alterations in the quantity and quality of ingested food influence thyroid function (Eales, 1988). Many studies demonstrated that the thyroid system is especially sensitive to carbohydrate content in diets (Harvey *et al*., 1981; Young *et al*., 1982, 1983; Danforth, 1986; Himick and Eales, 1990). Glucose in diet may stimulate the secretion of thyroid hormones (Hughes *et al*., 1984; Himick and Eales, 1990), especially T_4, or may act to enhance T_4 deiodination peripherally (Decuypere and Kuhn, 1984).

Alternatively, other nutritional components of diet, possibly protein, may be involved in the regulation of thyroid function. Lipid on the other hand, did not appear to modify thyroid function in rainbow trout under isocaloric conditions (Eales *et al*., 1990). The plausible function of dietary protein on the thyroid system may be related to the observations that food intake also influences T_4 5' monodeiodinase (which converts T_4 to T_3) as well as blood protein binding to circulating thyroid hormones (Eales, 1988).

Even though much research has been performed on the temperature related changes in plasma thyroid hormones, glucose, or protein (Hutton and Goodnight, 1957; Sellers *et al*., 1982; Bona-Gallo *et al*., 1984; Abdel-Raheem *et al*., 1989), the influence of nutritional status on those factors has rarely been investigated in reptiles. There have been a few experiments conducted on the effects of starvation on blood glucose or protein level in turtles (Bonnet, 1979; Da Silva and Migliorini, 1988). In freshwater turtles, starvation did not affect blood glucose level (Machado *et al*., 1989; Da Silva and Migliorini, 1988). However in sea turtles, starvation reduced blood glucose concentration and maintained constant protein level (Bonnet, 1979). From these studies, further research is suggested to develop a more detailed understanding of the effect of nutritional status on blood glucose and protein in reptiles. Additionally, thyroid hormone, as one of the metabolically important hormones in reptiles as well as in other classes of vertebrates, needs to be understood in relation to nutritional status. No documentation or data on thyroid-nutrition relationships in reptiles are available for this purpose. From previous study (Moon, 1992), it was found that plasma thyroid hormones decreased significantly in cold water. Either temperature or temperature and nutrition were suggested as the causes of the decrease in thyroid hormones, since sea turtles stopped feeding in cold water. To determine if thyroid hormones are influenced by nutritional status, the effect of nutritional manipulation on thyroid function of sea turtles need to be examined under constant temperature condition.

Therefore, the present study was undertaken to determine the effects of nutrient intake on plasma thyroid hormones, glucose, and total protein levels in sea turtles. To test the hypothesis that a decrease of food intake will lower plasma thyroid hormone, glucose, and total protein levels, the animals were first fasted and then refed thereafter. In a separate experiment, turtles were fed with increased amounts of food to satiate them. These studies may elucidate the metabolic adjustment of sea turtles to fasting periods in nature, a possible adaptation of sea turtles to the ocean environment in which long migrations are achieved away from feeding grounds

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(Owens, 1981).

MATERIALS AND METHODS

Laboratory-acclimated immature green (*Chelonia mydas*) and Kemp's ridley (*Lepidochelys kempi*) sea turtles (8 of each species) were used in this experiment. The green sea turtles were hatched in Florida in 1982 and raised in the laboratory for 8 years. The Kemp's ridley sea turtles had been hatched at Padre Island National Seashore, Texas, in 1987 and were obtained from the National Marine Fisheries Service Galveston Headstart Program. At the beginning of the experiment, the green turtles were 8 years old and 10–12 kg body weight (BW). The Kemp's ridley sea turtles were 3 years old and 11–15 kg BW. Each species was randomly divided into two groups of 4, experimental and control, which were put in separate 2,000 gallon artificial sea water raceways (7 m long by 1 m wide by 0.75 m deep) in two separate rooms of the Bioaquatic Facility of the Biology Department, Texas A & M University. Each turtle was kept isolated in its raceway by a plastic pipe divider. Constant water temperature of 25°C was maintained during the study. To examine the effects of fasting, the experimental group was fed diet every day at 4:00 PM for 15 minutes with turtle burgers made of Purina Trout Chow and a squid (twice a week), followed by 2 weeks of fasting (no feeding), then returned to the basal diet (turtle burgers). The control group, in contrast, was fed the same diet during the 5-week period. Blood samples (3 to 4 ml) were collected at 10:00 AM from the dorsal cervical sinus of each turtle as described by Owens and Ruiz (1980). The sampling times were: at 2 weeks after initiation of basal diet, at 24 hr after initiation of fasting, at 1 week of fasting, at 2 weeks of fasting, at 24 hr following the return to the basal diet, and at 1 week after return to the basal diet. The turtles then had a month for recovery after the fasting/refeeding experiment. During this period the turtles were fed routinely with the basal diet.

For the increased feeding experiment, the experimental groups were fed a basal diet for 2 weeks followed by 2 weeks of increased feeding, and finally 1 week back on the basal diet. During the increased feeding phase, these turtles were given as many burgers as they could consume within 15 minutes, three times a day. The approximate number of burgers consumed by each turtle was counted by observation after 15 min. The control groups were fed the basal diet throughout the experiment. Blood samples (3 to 4 ml) were collected from each turtle. Sampling times were: at 2 weeks after initiation of the basal diet, at 24 hr after increased feeding, at 1 week of increased feeding, at 2 weeks of increased feeding, at 24 hr after return to the basal diet, and at 1 week after return to the basal diet. Blood samples were centrifuged immediately and kept frozen at –70 to –80°C until analyzed for thyroid hormones (T_3 and T_4), glucose, and total protein. To see if there are diurnal changes in plasma thyroid hormones and blood glucose levels in sea turtles, seven serial blood samples were collected form the same turtles during a 2-day period. Bleeding times were: morning (8:30 AM), afternoon (4:30 PM), and midnight (00:30 AM). In the midnight, to eliminate the possible disturbance of turtles by illumination red filter-covered flashlight was used when the turtles were grabbed and bled.

Thyroid hormone radioimmunoassay

A thyroid hormone radioimmunoassay (RIA) (MacKenzie *et al.*, (1977), with modifications described by Denver and Licht (1988)) was used for quantifying plasma thyroxine (T_4) and triiodothyronine (T_3). An important modification was the use of 5% rather than 25% polyethylene glycol for precipitation of bound hormone with second antibody. To validate the assay, parallelism and recovery tests were conducted. Supplemented and non-supplemented samples appeared to parallel to the standard. The average recovery rate was 94±5%.

Blood glucose assay

Blood glucose levels were determined using a blood glucose test kit (Sigma colorimetric test kit No. 635). For the standard curve, 50 µl of glucose standard solution (No. 63500) was added. For unknowns, 50 µl of duplicate plasma was added to each tube. To each tube, 2.5 ml of toluidine reagent was added followed by shaking to mix. All tubes were put into a vigorously boiling water bath for exactly 10 min. Treated samples were read for absorbance at 635 nm using spectrophotometer (LKB NOVASPEC Biochrom 4049) within 30 min.

Blood total protein assay

Blood total protein level was determined using a protein test kit (Pierce colorimetric test kit No. 23236X). This protein test is based on the Bradford method that utilizes the absorbance shift in Coomassie Brilliant Blue G50 solution. The standard curve was prepared with 100 µl of BSA (bovine serum albumin) standard at the range of 20 to 1.25 g/dl. To sample tubes in duplicate, 100 µl of diluted plasma (1:100) in distilled water was added followed by 3.0 ml of protein assay reagent. After mixing, these samples were read for absorbance at 595 nm using the same spectrophotometer as above.

Statistical analysis

One-way ANOVA (repeated measures) was used to determine if there were significant changes in plasma thyroid hormone, glucose, and total protein levels over the experimental period. The significant differences were determined at the level of $p<0.05$. The paired *t*-test was used to determine if there are significant differences between control and experimental groups. The ANOVA and *t*-test were performed using the University SAS system at Texas A & M University.

RESULTS

Fasted/refeed experiment

Plasma thyroid hormones — The ANOVA showed significant differences ($p<0.05$) in plasma T_4 levels over the study period in green turtles. T_4 was reduced from 9.8 ± 1.1 (mean ± SE) to 6.7±1.3 ng/ml as green turtles were deprived of food during the first week (Fig. 1). In the second week of food deprivation, T_4 remained depressed as in the first week. When

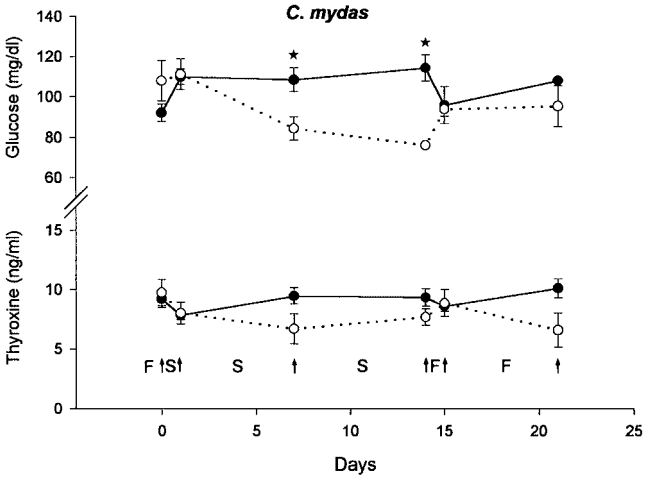

Fig. 1. Blood glucose (top) and T_4 (bottom) levels in green turtles, *Chelonia mydas*, in fast/refeed experiment. Vertical bars represent standard errors of the mean. The asterisks represent significant differences between control (solid line) and experimental (dotted line) groups by *t*-test. F: feeding, S: starving.

fasted turtles were refed, the concentration of plasma T_4 increased to 100% of control level within 24 hr. However, refed greens after one week had decreased again to 68% of control level. In the control group, plasma T_4 ranged from 7.9 ± 0.7 to 10.1 ± 0.8 ng/ml during the study, but did not vary significantly.

Fig. 2 shows the changes in plasma T_4 levels in fasted and refed ridley turtles. There were no significant differences between control and experimental groups. Plasma T_4 in the experimental group was consistently higher than in the control group except at one point at which T_4 decreased to lower than control group levels after refeeding for a week. This decrease by refeeding for 1 week was also observed in green turtles.

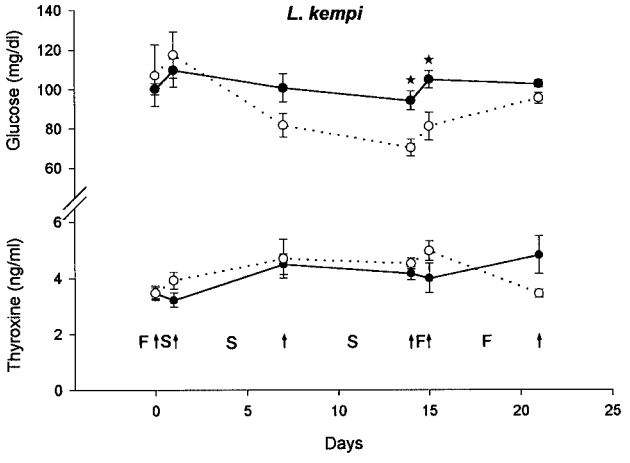

Fig. 2. Blood glucose (top) and T_4 (bottom) levels in Kemp's ridley turtles, *Lepidochelys kempi*, in fast/refeed experiment. Vertical bars represent standard errors of the mean. The asterisks represent significant differences between control (solid line) and experimental (dotted line) groups by *t*-test. F: feeding, S: starving.

Plasma T_3 levels were very low, ranging from 0.6 to 1.0 ng/ml (mean 0.8 ± 0.1 ng/ml) when green turtles were fed but decreased to 0.4 ± 0.1 ng/ml when they were fasted (Table 1). Any significant changes in T_3 by fasting were not observed even though significant differences existed between control and experimental groups at 24 hr and 1-week fasting. Plasma T_3 levels in Kemp's ridley turtles were low and relatively constant, always less than 0.5 ng/ml (Table 1). Table 2 shows the changes in the ratio of T_3 to T_4 during the period fasted. Both species did not show significant differences in the ratio of T_3/T_4.

Blood glucose — Blood glucose level in fasting green turtles varied significantly ($p<0.001$). There were no significant changes in blood glucose level after 1 day of starving, whereas continuous fasting for 1 week and 2 weeks decreased profoundly blood glucose level by 22% and 29%, respectively (Fig. 1). However, refed greens exhibited a rapid increase in glucose to the control level within 24 hr. Thereafter, glucose remained at about 90% of the control levels for the week of refeeding with a basal diet.

Kemp's ridleys presented a similar pattern in blood glucose changes relating to diet. Food deprivation decreased blood glucose level to 76% of initial level after the first week and to 64% after the second week, while refeeding increased glucose to 90% of the control level at the end of this experiment (Fig. 2).

Blood total protein — There were no significant effects of food deprivation on blood total protein levels in green turtles. Blood

Table 1. Plasma T_3 and total protein levels in green and Kemp's ridley turtles in fast/refeed experiment

	Species	Group	Day 0	1	7	14	15	21
T_3 (ng/ml) (mean±SE)	*C. mydas*	C	0.8 ±0.1	0.8 ±0.1	0.6 ±0.1	0.4 ±0.1	0.6 ±0.1	0.6 ±0.2
		E	1.0 ±0.3	0.3* ±0.1	0.2* ±0.0	0.4 ±0.1	0.5 ±0.2	0.9 ±0.2
	L. kempi	C	0.3 ±0.1	0.3 ±0.1	0.2 ±0.0	0.2 ±0.1	0.2 ±0.1	0.2 ±0.0
		E	0.2 ±0.1	0.2 ±0.1	0.2 ±0.1	0.2 ±0.1	0.3 ±0.1	0.2 ±0.1
Total Protein (g/dl) (mean±SE)	*C. mydas*	C	3.4 ±0.5	3.4 ±0.2	3.2 ±0.2	4.2 ±0.5	4.1 ±0.7	3.5 ±0.4
		E	3.7 ±0.1	3.8 ±0.5	3.3 ±0.2	4.2 ±0.5	3.8 ±0.6	4.0 ±0.9
	L. kempi	C	3.5 ±0.5	3.5 ±0.1	4.5 ±0.6	3.8 ±0.3	3.9 ±0.1	3.1 ±0.2
		E	3.5 ±0.6	4.1 ±0.3	4.7 ±0.4	3.7 ±0.1	3.9 ±0.2	3.7 ±0.7

C: control group E: experimental group
SE: standard error of the mean
The asterisks represent significant differences between control and experimental groups.

Table 2. Changes in the ratio of T_3/T_4 (%) in fast/refeed experiment

	C. mydas		*L. kempi*	
	C	E	C	E
basal diet	0.21	0.11	0.07	0.06
1 day fast	0.10	0.04	0.08	0.05
1 week fast	0.06	0.03	0.05	0.05
2 week fast	0.04	0.05	0.05	0.04
1 day refeed	0.07	0.05	0.06	0.05
1 week refeed	0.06	0.14	0.04	0.07

C : control group E : experimental group

protein ranged from 3.2 ± 0.5 to 4.2 ± 0.5 g/dl in greens during the study (Table 1). As in greens, blood protein in ridleys was not strongly affected by fasting. Individual variations and small number of animals reflected fluctuations in protein level.

Increased feed experiment

To determine the effect of increased feeding, greens and ridleys received about 254% of the basal diet (17.0 g/day/kg body weight) (data not shown). After feeding as much food as they could, the turtles either ignored or spit food out.

Plasma thyroid hormones — Plasma T_4 levels in green turtles decreased significantly as the turtles fed more ($p<0.05$). Within 1 day of increased feeding, greens recorded 30.0% increase in plasma T_4 level while consuming about 300% of the usual basal diet (Fig. 3). However, despite an average 200% increase in feeding over the 1 week, the T_4 decreased thereafter from 9.4 ± 0.7 ng/ml to 6.2 ± 1.3 ng/ml at day 7, which is a 34.7% decrease from the initial readings. Continual increased feeding returned T_4 to control levels, followed by a 22.9% increase within 1 day after returning to a basal diet. It is noticeable that there is a significant difference between control and experimental groups only at the first week of increased feeding.

In Kemp's ridley turtles, plasma T_4 levels followed a different pattern of change from greens in that after the first day of increased feeding T_4 decreased by 23.0% rather than increasing (Fig. 4). However as in greens, returning to a basal diet increased T_4 markedly in ridleys (40.1%).

Increased feeding did not affect significantly plasma T_3 in either species (Table 3). In greens, T_3 increased to 1.1 ± 0.5 ng/ml after 1 week of increased feeding and remained constant during the second week, but no significant differences between groups were found. In ridleys, the patterns of T_3 changes were not similar to those in greens and T_3 in experimental group remained relatively constant during the study period. The ratio of T_3 to T_4 changed significantly ($p<0.05$) by increased feeding in greens, whereas it did not in ridleys (Table 4).

Blood glucose — Unlike the food deprived/refeeding experiment, no significant changes in blood glucose levels with increased feeding were observed in either species (Figs. 3, 4). While the control group in greens maintained a stable glucose level, ranging from 91 ± 9.5 to 95 ± 4.7 mg/dl, during the study, ridleys showed relatively more variable glucose levels ranging from 84 ± 1.6 to 107 ± 6.5 mg/dl.

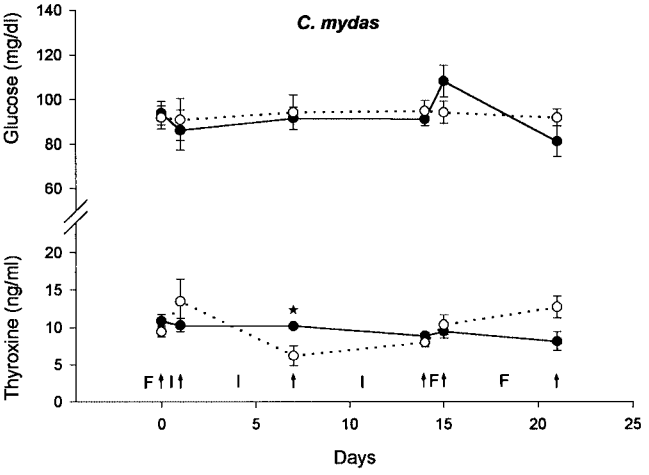

Fig. 3. Blood glucose (top) and T_4 (bottom) levels in green turtles, *Chelonia mydas*, in increased feed experiment. Vertical bars represent standard errors of the mean. The asterisk represents significant differences between control (solid line) and experimental (dotted line) groups by *t*-test. F: feeding, I: increased feeding.

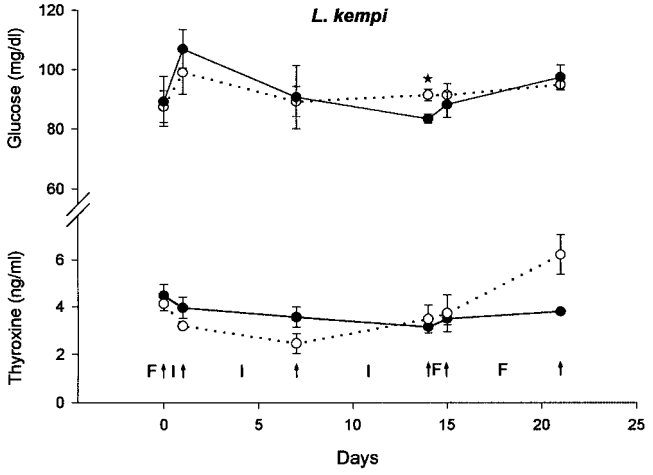

Fig. 4. Blood glucose (top) and T_4 (bottom) levels in Kemp's ridley turtles, Lepidochelys kempi, in increased feed experiment. Vertical bars represent standard errors of the mean. The asterisk represents significant differences between control (solid line) and experimental (dotted line) groups by *t*-test. F: feeding, I: increased feeding.

To determine if there are diurnal changes in plasma T_4 and blood glucose levels and to see the effects of multiple sampling for a short period on these, greens and ridleys were bled seven times repeatedly during a 2-day period. Fig. 5 shows diurnal changes in plasma T_4 and glucose, respectively. T_4 was variable but did not show significant changes in the morning, afternoon (after a meal), and midnight, ranging from 8.1 ± 0.7 to 10.5 ± 0.6 ng/ml in greens and 3.7 ± 0.4 to 4.6 ± 0.7 ng/ml in ridleys. Greens seemed to have more variable T_4 than ridleys, but no significant changes were found over a 2-day period (Fig. 5). No significant diurnal changes in blood

Table 3. Plasma T_3 and total protein levels in green and Kemp's ridley turtles in increased feed experiment

	Species	Group	Day 0	1	7	14	15	21
T_3 (ng/ml) (mean±SE)	*C. mydas*	C	0.7 ±0.3	0.7 ±0.0	0.6 ±0.1	0.6 ±0.1	0.7 ±0.1	0.8 ±0.2
		E	1.2 ±0.5	0.8 ±0.1	1.1 ±0.5	1.1 ±0.2	0.6 ±0.1	0.5 ±0.1
	L. kempi	C	0.2 ±0.1	0.3 ±0.1	0.3 ±0.0	0.4 ±0.1	0.2 ±0.0	0.3 ±0.1
		E	0.2 ±0.1	0.4 ±0.1	0.3 ±0.1	0.3 ±0.1	0.3 ±0.1	0.2 ±0.1
Total Protein (g/dl) (mean±SE)	*C. mydas*	C	3.0 ±0.5	3.2 ±0.1	4.2 ±0.8	3.2 ±0.1	3.3 ±0.5	3.7 ±0.3
		E	3.1 ±0.3	2.9 ±0.2	3.0 ±0.4	3.6 ±0.7	3.4 ±0.6	2.9 ±0.4
	L. kempi	C	3.4 ±0.4	2.8 ±0.2	2.5 ±0.2	2.7 ±0.2	3.2 ±0.3	3.1 ±0.4
		E	2.9 ±0.4	3.3 ±0.6	2.9 ±0.3	3.0 ±0.3	3.3 ±0.3	2.7 ±0.2

C : control group E : experimental group
SE: standard error of the mean

Table 4. Changes in the ratio of T_3/T_4 (%) in increased feed experiment

	C. mydas		*L. kempi*	
	C	E	C	E
basal diet	0.07	0.13	0.05	0.05
1 day satiated	0.07	0.07	0.09	0.12
1 week satiated	0.06	0.08	0.08	0.13
2 week satiated	0.07	0.14	0.13	0.09
1 day basal diet	0.07	0.06	0.06	0.10
1 week basal diet	0.09	0.04	0.07	0.04

C : control group E : experimental group

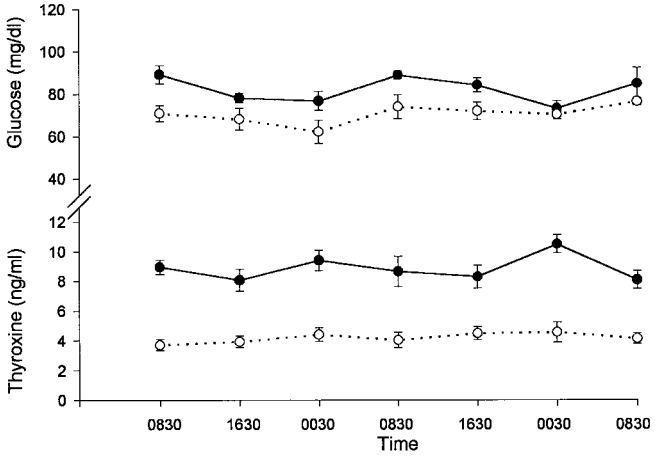

Fig. 5. Diurnal changes in blood glucose (top) and T_4 (bottom) levels in green (solid line) and Kemp's ridley (dotted line) turtles. Vertical bars represent standard errors of the mean.

glucose were observed in greens and ridleys. Blood glucose levels in ridleys were consistently lower (70.6±1.7 mg/dl) than those in greens (82.1±2.4 mg/dl) over a 2-day period. There seems no feeding or multiple sampling effect on glucose level.

Blood total protein — As in the fasted/refed experiment, no significant changes were observed in blood total protein levels in green or Kemp's ridley turtles (Table 3). Large standard error represented great individual variation in protein level. During the study, blood protein ranged from 3.0±0.5 to 4.2 ±0.8 g/dl in the control and 2.9±0.2 to 3.6±0.7 g/dl in the experimental group in greens, while protein in ridleys ranged from 2.5±0.2 to 3.4±0.4 g/dl in the control and from 2.7±0.2 to 3.3±0.3 g/dl in the experimental group.

DISCUSSION

The relationship between nutrition and thyroid hormones, or blood chemical components including glucose and protein has been investigated mainly in endothermic vertebrates, such as birds and mammals. In ectotherms, the most effort has been focused on salmonid fishes, whereas a very few studies have been conducted in reptiles. In the present study, nutritional effects on plasma thyroid hormones, glucose, and total protein levels were observed in captive greens, but not in Kemp's ridleys under constant temperature and photoperiod. In greens, plasma T_4 level changed significantly with deprivation of food. This finding agrees with that of Flood and Eales (1983) and Eales (1985) for salmonid fish. After an initial decrease during 1 week of fasting, T_4 level remained at a steady state which may be a result of decreased metabolic rate in green turtles as found in fish (Flood and Eales, 1983; Vijayan and Leatherland, 1989). In both species we found that when fasted turtles were refed, plasma T_4 concentration initially

increased but then decreased after a week. This long-term decrease may be explained by the increased conversion of T_4 to T_3 peripherally (Klandorf and Harvey, 1985). Therefore, more available T_3 would be produced from T_4 when the turtles receive more food. Unexpectedly, plasma T_4 also decreased as turtles consumed more food, as well as when they returned to a basal diet. This is inconsistent with a salmon study by McCormick and Saunders (1990) in which T_4 increased proportionally to increasing ration level. The possible factors which decrease plasma T_4 are increased clearance rate of T_4 in blood and increased secretion of T_4 from the gland (Eales, 1988). In the satiated state with increased diet, the clearance rate of T_4 in blood may be higher than thyroidal secretion rate of T_4 in turtles. Another possible factor is that the increased diet may induce an increase in T_3 secretion from the gland (Dauncey *et al.*, 1983). In both experiments, thyroid hormones appeared to be influenced by nutritional status within 24 hr. After this acute response to altered ration, the sea turtle thyroid system seemed to adjust to maintain baseline-circulating concentration of its hormones. In green turtles, T_4 returned to approximately 10 ng/ml after 2 weeks of fasting or increased feeding after an initial transient change, implying regulation of basal level around 10 ng/ml. It is assumed that decreased nutrients may cause decreased T_4 secretion that results in decreased conversion of T_4 to T_3. As greens take in more nutrients, there may be a transient increase in T_4 secretion, possibly followed by readjustment of the thyroid system through alterations in clearance rate. The thyroid system in ridleys appears more resistant to altered ration since basal T_4 levels were more variable than in greens. The reason that ridleys were different in their response is not certain, but genetic differences between two species may explain this discrepancy.

In the present study, blood glucose level was affected by fasting in sea turtles. The results agree with those in Bonnet's green turtle study (1979) in which blood glucose concentration dropped significantly as they starved for 5 days. Similar results were also found by Cherel *et al.* (1988) in birds. However, the present finding seems to contradict a freshwater turtle study by Da Silva and Migliorini (1988) in which no significant change was seen after fasting for 30 days followed by refeeding for 3 days. Da Silva and Migliorini (1988) found that maintenance of a constant glucose level in the blood was due to a high liver gluconeogenic activity for animals fed protein-rich and carbohydrate-poor diets. In fact, freshwater turtles were fed on a fresh meat diet, which possibly provided higher protein concentration than our pellet-diet for sea turtles. Similar results in which blood glucose level remained constant during fasting were found in mammals (Kettelhut *et al.*, 1980). In this study, cats and rats fed a protein-rich diet (63% protein) maintained a constant glucose level, whereas animals fed a carbohydrate-rich diet (70% carbohydrate) showed significantly lower glucose level than controls or animals fed the high-protein diet. Catfishes fed a carbohydrate-rich di*et al*so showed a progressive decline in blood glucose level as they were starved (Machado *et al.*, 1989). After 2 weeks of starvation, glucose reached about 80% of controls. In our turtles, either diet (protein content about 44%) or other internal factors, such as lower gluconeogenesis may have caused the decrease in blood glucose. Increasing the ration to 200% of the baseline did not cause any significant change in glucose, indicating that the liver or muscle acted as a reservoir for converting excess glucose into glycogen, as in refed fresh water turtles (Da Silva and Migliorini, 1988).

It was expected that stress, such as multiple sampling, might influence plasma thyroxine or glucose levels. In the present study, multiple sampling (7 times for 2-day period) did not affect on T_4 and glucose, indicating that changes in these are not likely caused by stress. Therefore, increased T_4 or glucose levels at 24 hr were not due to sampling on previous day. Blood total protein content did not change significantly during the study period, suggesting that blood protein in sea turtles may be under precise control during fasting, refeeding, and increased feeding. Despite no significant differences between groups, protein ranged from 2.9 to 4.2 g/dl and 2.5 to 4.7 g/dl in greens and ridleys, respectively. This range falls within the levels for other reptiles reported (Dessaeur, 1970); being similar to that in some chelonians, but lower than that for other reptilian species, including squamates and crocodilians (Cohen, 1954; Dessauer, 1970). Bonnet (1979) obtained similar results to the present study in that juvenile green turtles showed only slight variations in blood protein level during fasting. Changes in blood protein have been observed in several reptilian species. Most of this work revealed that blood protein level changes seasonally; lower in winter than summer. It can be assumed that during winter months animals do not feed well, resulting in low protein levels. In the present study, 2 weeks of fasting or increased feeding may not have been enough to observe substantial changes in serum protein level, since both greens and ridleys exhibited significant decreases in protein concentration when they stopped feeding when exposed to cold temperature for one or two months (Moon, 1992). This finding parallels that of Cherel *et al.* (1988) that fasted penguins maintained constant serum protein level for approximately 30 days and decreased thereafter. A protein sparing strategy such as this may be also rely on internal regulatory factors including hormones, such as thyroid hormones, insulin, or corticosterone (Cherel *et al.*, 1988; Vijayan and Letherland, 1989). Among these possible endocrine factors, thyroid hormones are known to activate protein synthesis in liver and muscle cells (Narayansingh and Eales, 1975; Medda and Ray, 1979). In contrast to anabolic effects, the catabolic effect of thyroid hormones on protein has also been seen in mammals and fish (Tata, 1974; Goldberg *et al.*, 1980). In our sea turtles, thyroid hormones tended to be negatively associated with protein as has been seen in lizard species (Mishra and Ahsan, 1988). The maintenance of a relatively constant protein level during starvation also can be seen in water snakes (Wasser, 1990) in which no seasonal variations in protein occurred. Glycogen or lipid may be possible factors to be utilized by sea turtles during short-term starvation as has been seen in hibernating snakes and mammals (Wasser, 1990) or other reptilian species (Derickson, 1976).

It may be hypothesized that our sea turtles use glucose from glycogen or lipid during the first phase of starvation for a month or so and thereafter they catabolize spared protein for the later phase of starvation. Reptiles, like fishes, are known to be able to survive prolonged periods of starvation, whether or not they hibernate (Bonnet, 1979). Starvation may induce a slower decline in glucose level so that the animals can prepare for long periods of starvation with a gradual decrease in glucose utilization (Machado *et al.*, 1989). From results of the experiments in which animals fed a high-protein diet maintained constant blood glucose level, it is assumed that the rate of glucose utilization may be related to protein content in a diet. Other factors involved in glucose metabolism in response to starvation or increased feeding are temperature, season, or sex (Sheridan and Mommsen, 1991). It is not known which one affects glucose metabolism in sea turtles. Whether they store protein, glycogen, or lipid, no doubt depends on the life history of the animals. Their activity and the usual duration of fasting may determine which nutrient system they mobilize to best advantage (Sheridan and Mommesen, 1991).

In summary, it is concluded that in sea turtles, nutritional status affects plasma thyroid hormones and glucose level, but not protein level. As for the thyroid function, plasma T_3 level and the ratio of T_3/T_4 did not change in consistent ways in relation to nutrition. Plasma T_3 tended to decrease during starvation and increase with refeeding or increased feeding, suggesting that deiodination of T_4 is facilitated by food intake (Eales, 1988). Further studies on deiodination activity in sea turtle thyroid system will be of interest. Blood glucose level falls with deprivation of food, but remains constant during increased feeding. No significant changes in blood protein level between control and experimental groups were observed under any nutritional condition in the present study. Sea turtles may use glycogen or lipid for energy during short-term starvation.

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