The Journal of Experimental Biology 203, 3631–3637 (2000)
Printed in Great Britain © The Company of Biologists Limited 2000
JEB2906

THYROID HORMONE CONCENTRATIONS IN CAPTIVE AND FREE-RANGING WEST INDIAN MANATEES (*TRICHECHUS MANATUS*)

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Accepted 12 September; published on WWW 2 November 2000

Summary

Because thyroid hormones play a critical role in the regulation of metabolism, the low metabolic rates reported for manatees suggest that thyroid hormone concentrations in these animals may also be reduced. However, thyroid hormone concentrations have yet to be examined in manatees. The effects of captivity, diet and water salinity on plasma total triiodothyronine (tT_3), total thyroxine (tT_4) and free thyroxine (fT_4) concentrations were assessed in adult West Indian manatees (*Trichechus manatus*). Free-ranging manatees exhibited significantly greater tT_4 and fT_4 concentrations than captive adults, regardless of diet, indicating that some aspect of a captive existence results in reduced T_4 concentrations. To determine whether this reduction might be related to feeding, captive adults fed on a mixed vegetable diet were switched to a strictly sea grass diet, resulting in decreased food consumption and a decrease in body mass. However, tT_4 and fT_4 concentrations were significantly elevated over initial values for 19 days. This may indicate that during periods of reduced food consumption manatees activate thyroid-hormone-promoted lipolysis to meet water and energetic requirements. Alterations in water salinity for captive animals did not induce significant changes in thyroid hormone concentrations. In spite of lower metabolic rates, thyroid hormone concentrations in captive manatees were comparable with those for other terrestrial and marine mammals, suggesting that the low metabolic rate in manatees is not attributable to reduced circulating thyroid hormone concentrations.

Key words: food restriction, lipolysis, metabolism, metabolic rate, thyroid hormone, marine mammal, manatee, *Trichechus manatus*.

Introduction

Manatees have been reported to exhibit metabolic rates as low as 25 % of that predicted on the basis of body mass (Scholander and Irving, 1941; Gallivan and Best, 1980, 1986). This low metabolic rate, together with their herbivorous feeding behavior (Campbell and Irvine, 1977; Best, 1981), makes them unique among marine mammals. The aquatic plants and algae these animals consume are 80–90 % water, resulting in a low energy density, providing a low caloric intake (Best, 1981) that may contribute to their reduced metabolic rate. The thyroid hormones triiodothyronine (T_3) and thyroxine (T_4) play a critical role in the regulation of basal metabolic rate in mammals (van Hardeveld, 1986), so the comparably low metabolic rates observed in captive manatees suggest that concentrations of thyroid hormones are also lower than in other mammals.

Because these hormones activate cellular metabolism and nutrient utilization, changes in circulating thyroid hormone concentrations can reflect changes in the rate at which an animal is undergoing energy-demanding processes such as fat mobilization (van Hardeveld, 1986; Eales, 1988). For example, in seals, thyroid hormones are thought to be important in regulating the rate of energy utilization and fat accumulation (Engelhardt and Ferguson, 1980; John et al., 1987; Renouf and Noseworthy, 1991). Thyroid hormones have also been reported to affect kidney function by increasing renal plasma flow, glomerular filtration rate and Na^+ transport (Capasso et al., 1999). Although manatees have been shown to consume large quantities of fresh water, some salt water is also ingested incidentally during feeding (Ortiz et al., 1999). Also, marine vegetation has a greater salt content than freshwater aquatic plants (Best, 1981), which may result in differences in renal handling of Na^+ in response to varying osmotic loads in the diet. Therefore, the ability of West Indian manatees to inhabit both fresh- and saltwater environments (Ortiz et al., 1998) suggests that thyroid hormone concentrations in manatees may be influenced by changes in water salinity. However, thyroid hormone concentrations in manatees have yet to be examined under any condition.

The present study was undertaken to examine circulating thyroid hormone concentrations in captive and free-ranging West Indian manatees (*Trichechus manatus*) under varying conditions. The objectives of the study were to compare concentrations between captive and free-ranging adults and to compare thyroid hormone concentrations from West Indian manatees in the present study with those previously reported for other mammals to determine whether thyroid function in manatees differs from that in other mammals or is affected by captivity. In addition, diet and salinity were manipulated in captive animals to examine their influence on concentrations of circulating hormones. This study may provide a better understanding of the role of the herbivorous diet of the manatee in influencing metabolism through thyroid hormone activity and whether thyroid hormone concentrations vary with captivity, diet or water salinity in a manner suggesting that these hormones function to promote energy-demanding processes.

Materials and methods

The present study was conducted with blood samples obtained during other physiological studies in West Indian manatees *Trichechus manatus* (L.) (Ortiz et al., 1998, 1999). Blood samples from both subspecies of the West Indian manatee, the Florida manatee (*T. m. latirostris*) and the Antillean manatee (*T. m. manatus*), were used in the present study. After the initial mention of each subspecies in the description of animals to follow, both subspecies will be referred to as West Indian manatees thereafter.

Concentrations of total triiodothyronine (tT_3) and thyroxine (tT_4) and of free thyroxine (fT_4) were compared among adult manatees on different diets and held or captured under varying environmental conditions (captivity and water salinity). A group of captive animals was sampled in October and again in January to determine whether temporal variation existed in hormone concentrations. The influence of captivity on hormone concentrations was examined by comparing levels between captive and free-ranging manatees. Also, the effects of reduced food intake and water salinity on hormone concentrations were examined in a group of captive animals that reduced their food intake when their diet was switched and in a separate group of captive animals that were switched between fresh, salt and fresh water.

Blood sampling procedures

For all captive manatees, blood samples were obtained by laying the animals on large foam mattresses on the bottom of their tank as water was drained from it. Once animals were situated on the foam mattresses, blood samples were collected within 10 min. All wild animals were captured with a net and stranded on shore. Their blood samples were obtained within 20 min of capture. One 10 ml heparinized Vacutainer (Becton-Dickinson, Rutherford, NJ, USA) was filled with blood from a vascular bundle near the plantar surface of the pectoral limb. Blood samples were immediately placed on ice until they could be centrifuged (10 min for 1500 ***g***). Plasma was transferred to cryovials (Nalge, Rochester, Ney, USA and Vangard, Neptune, NJ, USA) and frozen (at −20 °C or −70 °C) for later analyses.

Captive manatees

Blood samples were obtained from Florida manatees held in fresh water at Sea World of Florida (Orlando, FL, USA) in January (*N*=9; 150.0–972.7 kg) and October (*N*=4; 170.5–254.6 kg) 1993. The animals sampled in October were also used in the switched water salinity study (see below). Two animals (175.9 and 411.8 kg) held in fresh water at Lowry Park Zoo (Tampa, FL, USA) and four animals (>400 kg) held in salt water at EPCOT (Orlando, FL, USA) were also sampled in October 1993. Animals from the three parks were maintained on a diet of lettuce. Water temperature at the two parks is maintained between 24.5 and 26.5 °C throughout the year.

Six Antillean manatees (175.0–420.0 kg) held in salt water (34 ‰) at Centro Peixe-Boi in Itamaracá, Brazil, were sampled in February 1995. These animals were fed on a diet of mixed vegetables that included lettuce, cabbage, beets and carrots, supplemented with sea grass. These animals were also used in the study on the effects of reduced food intake (described below). Water temperature ranged between 25 and 26 °C during the study period.

Free-ranging manatees

All wild-caught animals were considered to be adults on the basis of straight length measurements (O'Shea et al., 1985).

Fresh water

Antillean manatees (*N*=9) were sampled in Colombia in July and August 1993. Animals were captured in a large freshwater lake and were feeding on native aquatic vegetation (Best, 1981).

Brackish water

Florida manatees (*N*=11) were captured at the freshwater effluent at Tampa Electric Company, Tampa Bay, FL, USA, in February 1993. Because these animals were caught in relatively low-salinity waters (<10 ‰; B. Weigle, Florida Department of Environmental Protection, personal communication), they were considered to be a brackish-water population. These animals were part of a population known to migrate between fresh- and saltwater habitats. Therefore, their diets were assumed to consist of both fresh- and saltwater vegetation (Campbell and Irvine, 1977; Lewis et al., 1984).

Salt water

Antillean manatees (*N*=5) were captured in a saltwater bay near La Ceiba, Puerto Rico, in May 1993. These animals were considered to be part of a marine population feeding on sea grasses and marine algae (Mignucci-Giannoni, 1998).

Food restriction study

Plasma samples were taken in conjunction with other physiological studies (Ortiz et al., 1998, 1999), so the sampling design had been established previously. The manatees held at Centro Peixe-Boi, Brazil, were serially sampled (on days 0, 3, 5, 7, 10, 14, 16 and 19) over a 19 day period. During this study, animals were switched from their normal diet of mixed vegetables to strictly sea grass (*Halodule* spp.) following the initial (day 0) blood sample. This switch in diet resulted in a decrease in food consumption leading to a decrease in body mass (Ortiz et al., 1999).

Switched water salinity study

Four manatees at Sea World were held in fresh water and maintained on lettuce. Animals were serially blood-sampled on days 0, 5, 10 and 15. Following the sample on day 5, the water in the animal's pool was switched to salt water (34 ‰) for 5 days before being switched back to fresh water.

Radioimmunoassays

Heparinized plasma was analyzed for tT_3, tT_4 and fT_4. Total thyroid hormone concentrations were measured by specific radioimmunoassay (RIA) for tT_3 and tT_4, as described previously (MacKenzie et al., 1993) and validated for manatee plasma. Curves for serially diluted plasma pools were parallel to the standard curve. Intra- and interassay variability (calculated as the coefficient of variation, CV, for at least five samples in three assays) for both assays was less than 9 %. Free T_4 was measured using a commercially available dialysis RIA kit (Nichols Institute, San Juan Capistrano, CA, USA). Intra- and interassay CV was less than 5 %. The lack of sufficient plasma sample from each animal in the switched water salinity study excluded measurements of fT_4 concentration in this group.

Statistical analyses

Means for each group were compared using analysis of variance (ANOVA). Means for animals in the food restriction and switched water salinity study were analyzed by ANOVA adjusted for repeated measures. A Fisher's PLSD was administered *post-hoc* if significant interactions (time or group) were observed. All statistical analyses were performed using StatView (SAS Institute, 1998). Values are reported as means (± S.E.M.), unless indicated otherwise, and were considered significantly different at $P<0.05$.

Results

Effects of temporal variation and captivity

For captive manatees sampled in October, thyroid hormone concentrations were not significantly different between animals held in fresh and salt water, so their values were pooled. Between January and October, tT_3 concentrations decreased significantly in captive manatees, while total and free T_4 concentrations did not change (Table 1). Captive manatees in Florida (regardless of month) had significantly lower tT_3 concentrations and significantly higher total and free T_4 concentrations than captive manatees in Brazil (Table 1). Free-ranging manatees (regardless of salinity) exhibited significantly lower tT_3 concentrations and significantly higher total and free T_4 concentrations than the captive groups, with three exceptions (Table 1).

Effects of reduced food intake

Animals reduced their food intake initially and lost between 2 and 17 % of their body mass over the first 5 days (Ortiz et al., 1999). Total T_3 concentrations did not change over this 19 day period; however, total and free T_4 concentrations were elevated by day 3 and day 5, respectively, and remained elevated over initial concentrations for the remainder of the study (Fig. 1).

Effects of switched water salinity

All animals ate normally and maintained their body mass throughout the 15 day period. Thyroid hormone concentrations were not altered when water was switched among fresh, salt and fresh water (Table 2).

Discussion

Manatees exhibit significantly lower metabolic rates than predicted for either terrestrial or other aquatic mammals (Scholander and Irving, 1941; Gallivan and Best, 1986; Worthy, 1990). Because thyroid hormones have been shown to play a critical role in the regulation of basal metabolic rate and oxygen consumption in mammals (van Hardeveld, 1986),

Table 1. *Total triiodothyronine (tT_3), total thyroxine (tT_4) and free T_4 (fT_4) concentrations for captive and free-ranging adult West Indian manatees*

Group	Location	*N*	Diet	Water salinity	$[tT_3]$ ($ng\,ml^{-1}$)	$[tT_4]$ ($ng\,ml^{-1}$)	$[fT_4]$ ($pg\,ml^{-1}$)
Captive	Florida						
January 1993		9	Lettuce	Fresh	$2.2±0.2^{a}$	$47.8±3.7^{a}$	$9.6±0.6^{a}$
October 1993		10	Lettuce	Fresh and salt	$1.6±0.1^{b}$	$41.8±3.5^{a}$	$11.3±0.9^{a,c}$
Captive	Brazil	6	Mixed vegetables	Salt	$2.8±0.3^{c}$	$19.0±2.5^{b}$	$5.2±0.7^{b}$
Free-ranging	Colombia	9	Aquatic plants	Fresh	$1.5±0.1^{b}$	$45.0±3.5^{a}$	$13.3±0.4^{c}$
Free-ranging	Florida	11	Both*	Brackish	$1.4±0.1^{b}$	$66.3±2.3^{c}$	$14.2±1.0^{c}$
Free-ranging	Puerto Rico	5	Marine vegetation	Salt	$1.6±0.2^{b}$	$82.7±4.9^{d}$	$15.9±1.3^{c}$

*Both refers to both freshwater and marine vegetation.
Values are means ± S.E.M. and were considered significantly different at $P<0.05$.
Common superscripts in each column indicate no significant difference.

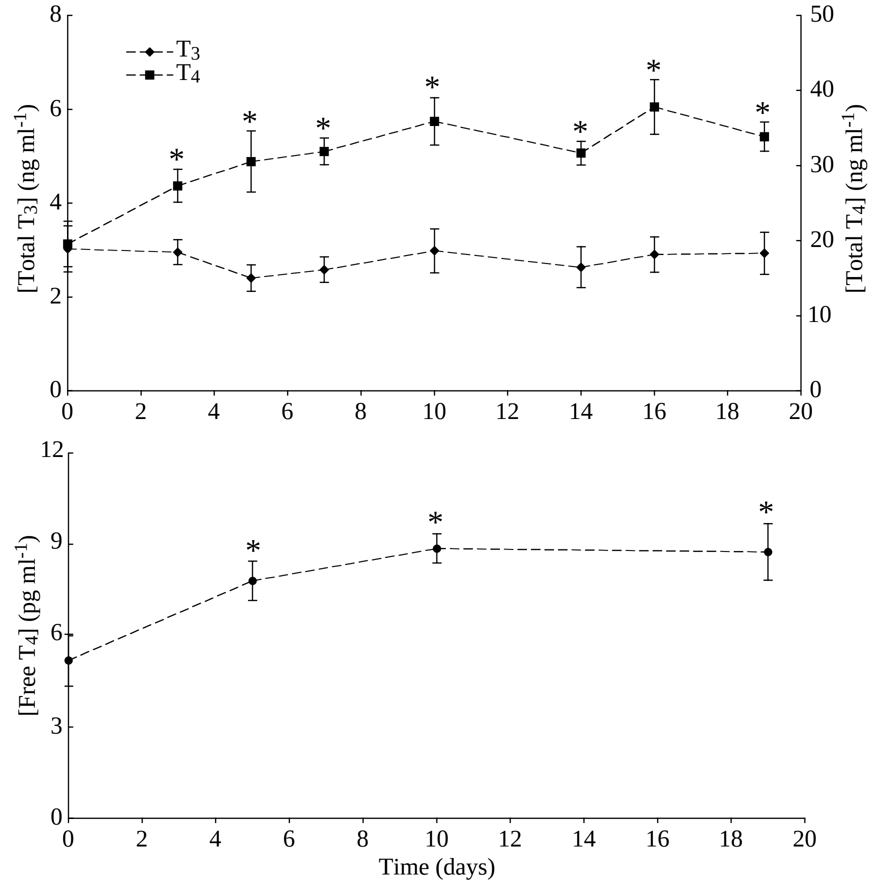

Fig. 1. Effects of reduced food intake on total triiodothyronine (T_3), total thyroxine (T_4) and free thyroxine (T_4) concentrations. Manatees (N=6) were switched from their normal diet of mixed vegetables to strictly sea grass after day 0, which resulted in a decrease in food intake and body mass. Means (± s.e.m.) significantly (P<0.05) different from day 0 values are marked with an asterisk.

we suspected that reduced metabolic rates in manatees might be reflected by comparably lower thyroid hormone concentrations. Also, levels of activity, food availability and kidney function have been associated with changes in thyroid hormone concentrations in mammals (Ringberg et al., 1978; Nilssen et al., 1984; St. Aubin and Geraci, 1988; Janan et al., 1995; St. Aubin et al., 1996; Capasso et al., 1999). Thyroid hormone concentrations in the present study represent the first published values for circulating levels in captive or free-ranging manatees. Values for tT_3, tT_4 and fT_4 determined for West Indian manatees in the present study are not exceptional, but are within the range of values reported for other marine mammals and for some terrestrial herbivores (Table 3). Therefore, the low metabolic rates reported for manatees (Scholander and Irving, 1941; Gallivan and Best, 1980, 1986; Miculka and Worthy, 1995) may not be attributable to reduced circulating thyroid hormone concentrations.

Circulating thyroid hormone concentrations represent just one component of the multilevel control of target tissue metabolism by the hypothalamic-pituitary-thyroid axis. Although circulating concentrations of thyroid hormones can be indicative of thyroid activation in an animal, T_4 must be enzymatically deiodinated to T_3 prior to binding nuclear receptors in order to exert thyroid-regulated effects (Oppenheimer et al., 1987; McNabb, 1992). Membrane transport, cellular deiodination and receptor availability and function are all components of the cellular actions of thyroid hormones that influence the potency of thyroid effects on target tissues (McNabb, 1992). Although it was not possible to evaluate all these aspects of thyroid function in the present study, total hormone concentrations were measured as an initial index of the activation of the pituitary-thyroid axis and of the available thyroid hormone blood pool. In addition, because it has been proposed that concentrations of free thyroid hormones provide a better index of thyroid hormone availability to activate intracellular processes (Ekins, 1986), free hormone measurements were made as an index of hormone supply to target tissue. Although these measurements did not reveal notable differences between manatees and other

Table 2. *Effects of switched water salinity on total triiodothyronine (tT_3) and total thyroxine (tT_4) concentrations in four West Indian manatees*

	Fresh water		Salt water	Fresh water
	Day 0	Day 5	Day 10	Day 15
[tT_3] (ng ml^{-1})	1.5±0.1	1.5±0.3	1.4±0.1	1.4±0.1
[tT_4] (ng ml^{-1})	39.8±3.8	43.1±6.5	44.0±5.4	42.4±5.0

No significant differences were observed.

Values are means ± s.e.m.

*Animals were maintained in fresh water between days 0 and 5, in salt water between days 6 and 10, and returned to fresh water between days 11 and 15.

Table 3. *Comparison of thyroid hormone concentrations among various terrestrial herbivores and other marine mammals*

	Mass (kg)	Condition	[tT_3] ($ng\,ml^{-1}$)	[tT_4] ($ng\,ml^{-1}$)	[fT_4] ($pg\,ml^{-1}$)	Source
Terrestrial						
Svalbard reindeer	53±1	SD	1.3–2.6*	NR	39–101*	Nilssen et al. (1984)
Norwegian reindeer	68±2	SD/FR	1.3–2.2*	122–173[1]	98–117*	Nilssen et al. (1984)
Dairy cows	NR	Captive	1.6±0.2	50±2	NR	Bitman et al. (1994)
Angus-Hereford steer	302±16	Captive	2.2±0.1	75±2	NR	Kahl et al. (1992)
Holstein-Friesian bull	945±15	Captive	0.3*	120*	NR	Janan et al. (1995)
Marine						
Polar bear	NR	FR	0.7–1.5	32–52	NR	Leatherland and Ronald (1981)
Harbor seal	NR	Captive	0.2–1.4*	10–40*	10–70*	Ashwell-Erickson et al. (1986)
Harbor seal	NR	Captive	0.5±0.1	29±1	16±1	Renouf and Brotea (1991)
Harp seal	136–150	FR	2.0±1.1	25±15	NR	Engelhardt and Ferguson (1980)
Harp seal	NR	Captive	1.7±0.3	45±11	NR	John et al. (1987)
Gray seal	NR	FR	1.1±0.8	16±3	NR	Engelhardt and Ferguson (1980)
Bottlenose dolphin	NR	SD/FR	0.8–2.6	85–242	7–21	St. Aubin et al. (1996)
Beluga whale	NR	FR	1.8±1.1	194±23	NR	St. Aubin and Geraci (1988)
Beluga whale	NR	Captive	1.0±0.5	93±27	NR	St. Aubin and Geraci (1988)
Pilot whale	390–520	SD/FR	NR	43±16	NR	Ridgway and Patton (1971)
Manatee	150–900	Captive	1.9–2.8	19–45	5–11	This study
Manatee	NR	FR	1.4–1.6	45–83	13–16	This study

tT_3, total triiodothyronine; tT_4, total thyroxine; fT_4, free thyroxine.
*Values extrapolated from figures; [1]extrapolated from Ringberg et al. (1978).
SD, semidomesticated; FR, free-ranging; NR, not reported. Means ± S.D. or ranges are reported.

mammalian species, they did provide evidence that thyroid activity differs among populations of manatees. In the present study, captivity was associated with reduced total and free T_4 concentrations, whereas reduced food intake and a diet that included marine vegetation were associated with increased total and free T_4 levels. Water salinity, however, did not appear to influence concentrations of circulating thyroid hormones in manatees.

Although manatees exhibit metabolic rates lower than that predicted by body mass under normal conditions, they have been reported to reduce their metabolic rate by an additional 23 % during fasting (Gallivan and Best, 1986). In general, fasting and food restriction decrease both T_3 and T_4 levels in most mammals (Eales, 1988; Yen et al., 1994; Janan et al., 1995). For example, fT_4 concentrations were positively correlated with food intake in harbor seals (*Phoca vitulina*) (Renouf and Noseworthy, 1991). In the present study, captive adults, deprived of their normal vegetable diet and provided with only sea grass, reduced their food intake, which was associated with a decrease in body mass of as much as 17 % (Ortiz et al., 1999). However, free and total T_4 concentrations were significantly elevated despite this reduction in food intake. Ortiz et al. (1999) suggested that the observed loss of body mass during the first 5 days was primarily fat which, when oxidized, accounted for the entire water turnover rate of these animals. In the present study, free and total T_4 concentrations plateau after the fifth day, when the animals stopped losing weight (Ortiz et al., 1999). Therefore, it would appear that the increase in total and free T_4 concentrations may be associated with an increase in lipolytic activity (Cheikh et al., 1994), which may be adaptive since marine mammals are known to utilize the water from fat oxidation to maintain fluid balance during periods of food restriction (Ortiz et al., 1978).

Alternatively, the observed increases in both total and free thyroxine concentrations may not reflect increased synthesis and release, but rather altered binding kinetics of the free fraction, decreased clearance from the circulation or decreased deiodinase activity. In hibernating ground squirrels (*Spermophilus richardsoni*), an increase in total thyroid hormone concentrations was attributed to an increase in binding capacity and affinity since the percentage of free hormone was reduced (Magnus and Henderson, 1988). However, in the present study, total and free thyroxine concentrations increased proportionally (i.e. in the same ratio), suggesting that binding capacity was not increased as it was in hibernating squirrels. In torpid squirrels, the increase in thyroxine concentrations was attributed to greatly reduced clearance rates (Demeneix and Henderson, 1978), which may be a possibility in the present study. Fasting may also induce an increase in deiodinase activity (Diano et al., 1998); however, in the present study, tT_3 concentrations remained constant while tT_4 concentrations were elevated, suggesting that deiodinase activity had decreased. Regardless, the presence of elevated thyroid hormone concentrations in the circulation may still permissively support fat metabolism *via* their lipolytic functions.

Aside from food availability, the level of activity, which may be reflected by captivity in some animals, may also affect thyroid hormone concentrations. Free-ranging beluga whales (*Delphinapterus leucas*) maintained in captivity for 10 weeks exhibited significantly reduced tT_3 and tT_4 concentrations (St. Aubin and Geraci, 1988). A decrease in activity associated with captivity was also correlated with a decrease in thyroid hormone concentrations in reindeer (*Rangifer tarandus tarandus*; Ringberg et al., 1978). The observation that free-ranging manatees exhibited greater total and free T_4 concentrations than captive manatees suggests that some aspect of a free-living existence, possibly increased activity or diet, resulted in increased thyroid hormone concentrations in wild populations. Captive adults maintained on either lettuce or mixed vegetable diets had generally lower total and free T_4 concentrations than free-ranging animals, suggesting that captivity may have contributed to the reduced thyroid hormone concentrations. The variability in thyroid hormone concentrations among the free-ranging groups may reflect the variety of natural vegetation being consumed.

Manatees in salt and brackish water are known to consume sea grasses along with marine algae (Lewis et al., 1984; Mignucci-Giannoni, 1998), which may contain large quantities of iodine (Devi et al., 1996). Because elevated dietary iodine concentrations may promote increased thyroid hormone concentrations (Knudsen et al., 1999), the higher tT_4 concentrations measured in the free-ranging manatees, which are known to consume marine algae (Lewis et al., 1984; Mignucci-Giannoni, 1998), might be attributable to higher dietary iodine levels. Marine algae have been reported to contribute approximately 4 % to the diet of manatees around Puerto Rico (Mignucci-Giannoni, 1998), where our blood samples for free-ranging, saltwater manatees were obtained. Therefore, the lower thyroid hormone concentrations in captive animals may be due in part to an iodine-deficient diet. Also, if T_4 concentrations, especially fT_4 concentrations, provide a better indication of metabolic status than T_3 concentrations (Ekins, 1990), then our data would suggest that free-ranging animals have a higher metabolic rate than captive animals.

Although thyroid hormones are known to affect kidney function (Capasso et al., 1999), no association between water salinity and hormone concentrations were observed in the present study. The absence of a change in thyroid hormone concentrations when animals were switched among fresh, salt and fresh water together with the absence of consistent differences in concentrations among the free-ranging animals sampled in fresh, brackish and salt water suggest that changes in water salinity do not influence circulating thyroid hormone concentrations in manatees.

In summary, thyroid hormone concentrations measured in both captive and free-ranging manatees are within the ranges reported for other marine mammals and terrestrial herbivores. Reduced food consumption in captive manatees resulted in an elevation of total and free T_4 concentrations, which may have potentiated lipolysis in these animals in an effort to maintain water balance during periods of food deprivation. Some aspect of a free-ranging existence, possibly increased activity, appears to have a greater influence on total and free T_4 concentrations than diet, as indicated by the increased concentrations in wild animals, regardless of their diet. However, the variability in thyroid hormone concentrations among the free-ranging animals may reflect the differences in iodine content of the natural vegetation consumed. Changes in water salinity did not alter thyroid hormone concentrations in the present study. Also, if thyroid hormone concentrations, especially fT_4 concentrations, are an indication of metabolic status, then free-ranging manatees would appear to have greater metabolic rates than captive manatees.

Research was funded by EPCOT's Living Seas, Florida Department of Environmental Protection, Save the Manatee Club, Sea World of Florida and the US Fish and Wildlife Service. We would like to thank the following for providing samples from free-ranging manatees: B. Bonde, A. Mignucci, R. Montoya, B. Weigle and S. Wright. We thank the Caribbean Stranding Network, Centro Peixe-Boi/IBAMA, EPCOT's Living Seas, Lowry Park Zoo and Sea World of Florida for their assistance and for allowing us to work at their facilities. From Texas A&M University, we thank T. Christopher, T. Miculka and M. Reimers for their assistance. This research was authorized under USFWS permit 766146 to G.A.J.W. and was approved by the Texas A&M University Animal Care and Use Committee.

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