Original investigation

Seasonal response of ghrelin, growth hormone, and insulin-like growth factor I in the free-ranging Florida manatee (*Trichechus manatus latirostris*)

Rachel L. Tighe [a], Robert K. Bonde [b], Julie P. Avery [a],*

[a] *University of North Florida, Department of Biological Sciences, 1 UNF Drive, Jacksonville, FL 32224, USA*
[b] *U.S. Geological Survey, Southeast Ecological Science Center, 7920 NW 71st Street, Gainesville, FL 32653, USA*

ABSTRACT

Seasonal changes in light, temperature, and food availability stimulate a physiological response in an animal. Seasonal adaptations are well studied in Arctic, Sub-Arctic, and hibernating mammals; however, limited studies have been conducted in sub-tropical species. The Florida manatee (*Trichechus manatus latirostris*), a sub-tropical marine mammal, forages less during colder temperatures and may rely on adipose stores for maintenance energy requirements. Metabolic hormones, growth hormone (GH), insulin-like growth factor (IGF)-I, and ghrelin influence growth rate, accretion of lean and adipose tissue. They have been shown to regulate seasonal changes in body composition. The objective of this research was to investigate manatee metabolic hormones in two seasons to determine if manatees exhibit seasonality and if these hormones are associated with seasonal changes in body composition. In addition, age related differences in these metabolic hormones were assessed in multiple age classes. Concentrations of GH, IGF-I, and ghrelin were quantified in adult manatee serum using heterologous radioimmunoassays. Samples were compared between short (winter) and long (summer) photoperiods ($n = 22$ male, 20 female) and by age class (adult, juvenile, and calf) in long photoperiods ($n = 37$). Short photoperiods tended to have reduced GH ($p = 0.08$), greater IGF-I ($p = 0.01$), and greater blubber depth ($p = 0.03$) compared with long photoperiods. No differences were observed in ghrelin ($p = 0.66$). Surprisingly, no age related differences were observed in IGF-I or ghrelin concentrations ($p > 0.05$). However, serum concentrations of GH tended ($p = 0.07$) to be greater in calves and juveniles compared with adults. Increased IGF-I, greater blubber thickness, and reduced GH during short photoperiod suggest a prioritization for adipose deposition. Whereas, increased GH, reduced blubber thickness, and decreased IGF-I in long photoperiod suggest prioritization of lean tissue accretion. Hormone profiles in conjunction with difference in body composition between photoperiods indicate seasonal adjustments in manatee nutrient partitioning priorities.

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Introduction

Mammals live in diverse environments and are confronted with consistent seasonal environmental fluctuations in light, temperature, and food availability. Seasonal environmental changes may stimulate a physiological response in an animal often initiated by the endocrine system to maintain homeostasis (Florant and Healy, 2012). Environmental stimuli may elicit a response of the organism to adjust homeostatic set points. These adjustments result in acclimatization and may include responses, such as

alterations in food intake, body weight, fat content, and hormonal functions (Florant and Healy, 2012). Metabolic hormones such as ghrelin, growth hormone (GH), and insulin-like growth factor (IGF)-I function to regulate tissue-specific energy allocation and together link growth physiology, developmental age, energy balance (the amount of calories consumed versus metabolized and stored), and nutritional status (negative [fasting] or positive nutrient intake) in domestic animals (Bergan et al., 2015; Breier, 1999; Cammisotto et al., 2010; Castaneda et al., 2010; Lawrence and Fowler, 1997).

The secretion of the metabolic hormone ghrelin in the stomach is influenced by nutritional status (Cummings et al., 2001; Salbe et al., 2004). The increase of ghrelin during fasting or reduced nutrient intake stimulates the release of GH, fat oxidation, and food intake, and inhibits body weight gain and adiposity (Tschop et al., 2000). Growth hormone regulates energy deposition and mobilization through its direct action on adipose tissue (Bergan et al., 2015;

Abbreviations: GH, growth hormone; IGF, insulin-like growth factor.
* Corresponding author.
E-mail addresses: RachelLynnTighe@gmail.com (R.L. Tighe), rbonde@usgs.gov (R.K. Bonde), j.avery@unf.edu (J.P. Avery).
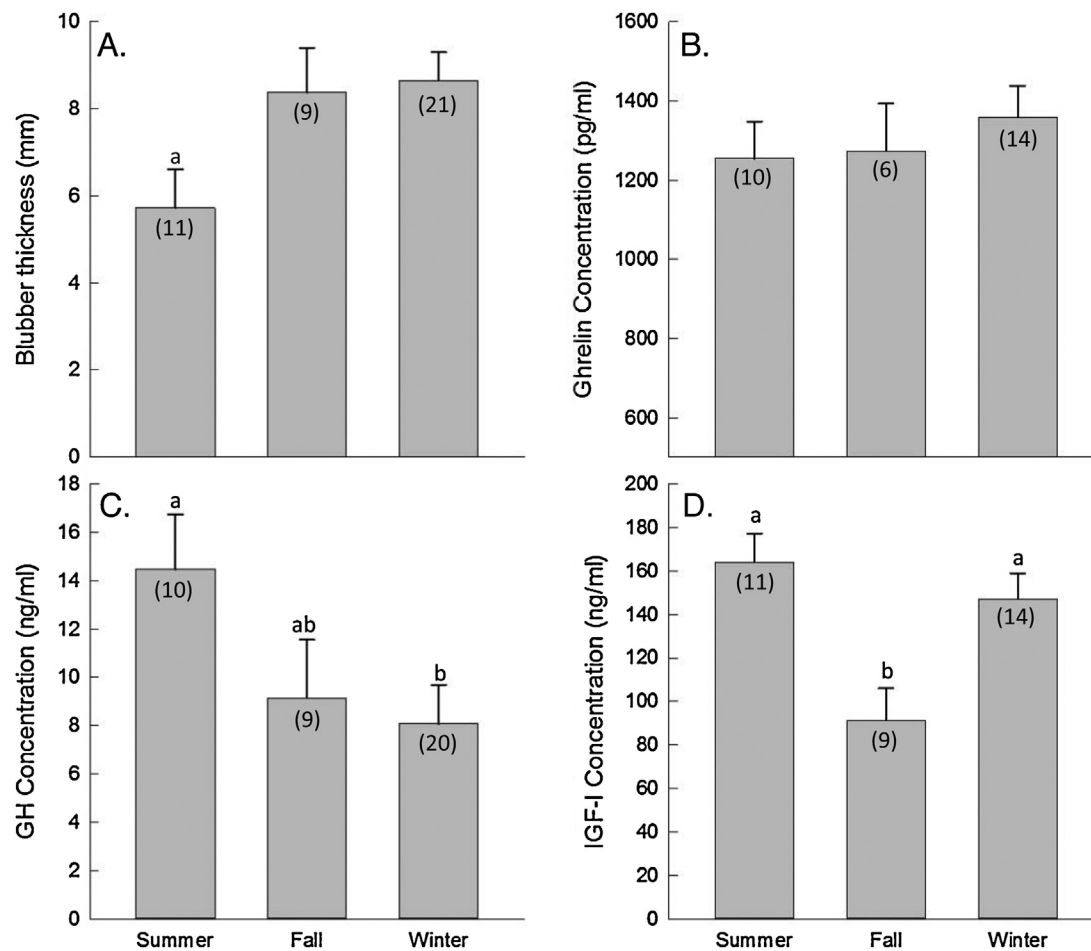
Fig. 1. (A–D) Pattern among seasons (summer, fall, winter) presented as mean ± SE in adult free-ranging manatees from 2005 to 2010. (A) Average blubber thickness (mm) (B) Average ghrelin concentrations (pg/ml) (C) Average GH concentrations (ng/ml) (D) Average IGF-I concentrations (ng/ml). Significant differences among seasons were presented as letters (a, b). Sample size is within the column of each season.

Breier, 1999). During energy deficiency or periods of low nutritional status, GH stimulates lipolysis and inhibits lipogenesis (Bergan et al., 2015). The mobilization of fat from adipose tissue, or lipolysis, provides energy for maintenance (Vijayakumar et al., 2010). When nutritional intake is adequate for energy requirements of maintenance, GH stimulates the production and release of IGF-I from the liver. Insulin-like growth factor-I regulates somatic growth, cell proliferation, differentiation, and apoptosis (Renaville et al., 2002). During normal physiological conditions IGF-I stimulates protein synthesis, bone formation, and glucose uptake (Etherton, 1991).

Modifications in nutrient intake or seasonal abiotic variables elicit an endocrine response resulting in alteration of growth rate and tissue-specific nutrient priorities (Gerrard and Grant, 2006). Mammals that exhibit seasonal changes in body composition are generally leaner during long photoperiod (summer, greater than 12 h of daylight) with greater adiposity during the short photoperiod (winter, less than 12 h of daylight; Florant and Healy, 2012; Webster et al., 1999; Zhang et al., 2012; Zhu et al., 2012; Zinn et al., 1986). Some mammals demonstrate weight gain, with increased adiposity, during a transitional period (fall) in preparation for the winter when food may be scarce, ambient temperature declines, and additional energy is needed for thermoregulation (Florant and Healy, 2012; Fuglei et al., 2004; Webster et al., 1999; Zhang et al., 2012; Zhu et al., 2012). During winter, some mammals minimize lean tissue accretion to conserve body protein (Nunes et al., 1998; Prestrud and Nilssen, 1992; Webster et al., 1999). Previous studies found that during winter ghrelin stimulates lipogenesis and food

intake (Tschop et al., 2000). During fall GH was reduced to facilitate adipose accumulation in preparation for short photoperiod (Fuglei et al., 2004). In contrast, summer GH concentrations were increased to facilitate lean tissue accretion mediated by IGF-I (Rosenfeld and Hwa, 2009). In previous studies of ungulates, IGF-I concentrations were greater in summer compared with winter and were correlated with increased lean tissue accretion (Barenton et al., 1988; Comeau et al., 2008; Webster et al., 1996).

Most research on the role of metabolic hormones in the regulation of seasonal change in body composition has been examined in species living at middle to high latitudes that exhibit strong seasonal patterns in food intake and body composition (Fuglei et al., 2004). However, the physiological mechanism for seasonal body composition is unknown in sub-tropical or tropical species with less pronounced seasonal patterns. The free-ranging Florida manatee (*Trichechus manatus latirostris*), a subtropical aquatic mammal, seasonally shift their habitat preference due to their narrow thermoneutral zone (Reynolds and Wilcox, 1986). Manatees tend to remain relatively inactive in warm water areas during cold air or ambient temperatures to reserve energy (Reynolds and Rommel, 1996; Reynolds and Wilcox, 1986). Therefore, manatees feed and forage less frequently resulting in negative energy balance and blubber loss (Gallivan et al., 1983; Reynolds, 2000). While seasonal changes in blubber thickness have been observed, the physiological mechanisms that contribute to this seasonal difference in body composition has not been explored in the Florida manatee. The primary objective of this research was to determine if manatee

Table 1

Sample size of manatee that were used to quantify hormone concentrations during photoperiod comparisons from Crystal River and Everglades, Florida from 2005 to 2010.

Age class[a]	Winter		Summer		Fall	
	Male	Female	Male	Female	Male	Female
Adult	11	10	9	3	2	7
Juvenile	6	3	—	—	—	—
Calf	5	3	—	—	—	—

[a] Age classifications were determined based on straight length measurements and defined as adult >260 cm, juvenile 236–260 cm, calf <236 cm.

exhibit a seasonal physiological response and determine if changes in ghrelin, GH, and IGF-I were related to changes in seasonal change in body composition of free-ranging Florida manatee. In addition, to determine if manatees exhibit the typical mammalian developmental pattern in these metabolic hormones, hormone concentrations were assessed in three different age classes of male manatee.

Material and methods

Sample collection

Research was conducted as part of routine manatee health assessments performed by the U.S. Geological Survey (USGS) Sirenia Project and the Florida Fish and Wildlife Conservation Commission (FWC) from 2005–2010. If individual manatees were captured in multiple photoperiods, only data collected in one photoperiod was used for this study. Age class was categorized for 59 manatees (33 male; 26 female) by standard straight-line length (tip of snout to tip of paddle), as follows: adult >260 cm; juvenile 236–260 cm; and calf <236 cm (Table 1; Bonde et al., 2012). During long photoperiod (summer; June–August) and the transitional period (fall; October), samples were collected from Everglades, Florida using nets in an open-water boat capture method (Wong et al., 2012). Samples during short photoperiod (winter; December–February) were collected from Crystal River, Florida using nets in a land based capture method (Bonde et al., 2012). During land and boat based captures, a No. 30 or 56 braided seine twine net 122 m long and up to approximately 8 m deep, with a 10- to 17-cm stretch knotted nylon mesh was deployed from a motorized modified mullet skiff with a removable transom. The net encircled the manatee, and the capture team pulled the net and the manatee onto the capture boat or river bank (Bonde et al., 2012; Wong et al., 2012). Manatees were then transported to the health assessment base on-shore for a medical examination by a team of biologists and veterinarians.

Blood handling and hormonal analyses

Blood samples were collected within 20 min of capture from the brachial bundle on the medial aspect of the pectoral flipper (Bonde et al., 2012; Stamper and Bonde, 2012) using serum separator tubes, and stored in a cooler with ice until processing (Bonde et al., 2012; Bossart et al., 2001; Stamper and Bonde, 2012). Within 1 h of collection, whole clotted blood was centrifuged at $6000 \times g$ for 10 min. Serum was collected and archived frozen at $-80\,°C$. A physical exam was conducted which included general appearance, body temperature, body mass, morphometrics (standard length and axillary girth), and blubber thickness measured via ultrasound (Bonde et al., 2012; Ward-Geiger, 1997).

Ghrelin concentrations were determined by a heterologous radioimmunoassay (RIA) kit (GHRT-89HK, Millipore, Billerica, MA). Manufacturer protocols were precisely followed with one modifi-

cation: all reagent and sample volumes were halved. Radioactivity of the final pellet was measured using a gamma counter (Wizard Walla 1470, Perkin Elmer, MA). Serum concentrations of GH and IGF-I were determined by heterologous RIA (Richmond and Zinn, 2009). Antisera and standards were purchased from A.F. Parlow, National Hormone & Peptide Program (Torrance, CA). Rabbit-anti-porcine antisera for GH (AFP422801) was used with goat secondary antisera against rabbit γ-globulin and porcine GH (AFP10864B) standard to quantify manatee GH concentrations. Serum concentrations of IGF-I were quantified with a rabbit-anti-human IGF-I antibody (AFP4892898) and purified human IGF-I standard (Lot #1). Serially diluted pooled serum from free-ranging West Indian manatees was used to validate heterologous ghrelin, GH, and IGF-I RIA. Standard validation parameters including percent recovery, precision of the assay, parallelism, and dilution linearity were assessed (Richmond and Zinn, 2009).

Statistical analysis

The homogeneity of variance (HOV) was calculated using the Bartlett test to determine normality and variance of hormone and morphometric measures between seasons, sex, and age classes. To evaluate seasonal differences, measurements of serum ghrelin, GH, and IGF-I and blubber thickness were analyzed in a general linear model using an analysis of variance (ANOVA) to determine significant differences (SAS Inst. Inc., Cary, NC). Due to the small sample size (Table 1) sex differences were not statistically tested. A Fisher's Least Significant Difference (LSD) post-hoc test was used to determine differences among seasons (summer, fall, winter). To test the effect of age, ghrelin, GH, and IGF-I concentrations were analyzed using an ANOVA in one season. The LSD post-hoc test was used to determine differences among age classes (calf, juvenile, adult). The results were presented as the mean ± standard error (SE). Differences were considered significant at p-value ≤ 0.05 and a trend if $0.05 < p$-value ≤ 0.10.

Results

Radioimmunoassay validations

Percentage recovery of pooled manatee serum spiked with human ghrelin standard RIA was $105.7 \pm 9.8\%$. Dilution series of pooled manatee serum was parallel to the ghrelin standard curve (dilution linearity $R^2 = 0.97$). The intra- and inter-assay coefficient of variation (CV) for ghrelin were 10.0 and 8.3, respectively. Percentage recovery of pooled manatee serum spiked with porcine GH standard RIA was $92.5 \pm 7.9\%$. Dilution series of pooled manatee serum was parallel to the GH standard curve (dilution linearity $R^2 = 0.98$). The intra- and inter-assay CV were 6.3 and 15.6, respectively. Percentage recovery of pooled manatee serum spike with human IGF-I standard RIA and high and low manatee serum was $93.1 \pm 5.7\%$. Dilution series of pooled manatee serum was parallel to the IGF-I standard curve (dilution linearity $R^2 = 0.95$). The intra- and inter-assay CV were 10.8 and 10.9, respectively.

Seasonal differences

As expected, blubber thickness differed between seasons ($p = 0.03$; Fig. 1A). Summer manatees had reduced blubber thickness (5.7 ± 0.9 mm) compared with the winter (8.7 ± 0.7 mm) and fall (8.4 ± 1.0 mm) animals. However, no difference was observed in blubber thickness between fall and winter seasons ($p = 0.82$). Ghrelin concentrations (Fig. 1B) were similar across all seasons ($p = 0.66$): summer (1255.1 ± 93.2 pg/ml), fall (1272.8 ± 120.4 pg/ml), and winter (1359.2 ± 78.8 pg/ml). There was a seasonal trend in GH concentrations ($p = 0.08$). Summer

Table 2
Reported results for ghrelin, GH, and IGF-I validations for manatee serum including dilution linearity, percentage recovery, and intra-assay and inter-assay coefficient of variation (CV).

Hormone	Dilution linearity	Percentage recovery[a]	Intra-assay CV[a]	Inter-assay CV[a]
Ghrelin (pg/ml)	$y = 30.383x - 90.963 R^2 = 0.97$	105.7 ± 9.8	9.9 ± 139.0	8.3 ± 110.2
GH (ng/ml)	$y = 12.303x + 11.385 R^2 = 0.98$	92.5 ± 7.9	6.3 ± 0.9	15.6 ± 2.5
IGF-I (ng/ml)	$y = 3.0958x + 0.7416 R^2 = 0.95$	93.1 ± 5.7	12.8 ± 10.1	10.3 ± 25.0

[a] Values reported as mean ± Standard Deviation.

had greater GH concentrations (14.5 ± 2.3 ng/ml) compared with winter at (8.1 ± 1.6 ng/ml; $p = 0.03$; Fig. 1C) while there were no differences between fall (9.1 ± 2.4 ng/ml) and either summer or winter ($p = 0.12$ or 0.71, respectively). Concentrations of IGF-I were different among seasons ($p < 0.01$; Fig. 1D) with greater concentrations during summer (164.0 ± 13.2 ng/ml) and winter (147.2 ± 11.7 ng/ml) compared with fall (91.3 ± 14.6 ng/ml). There were no differences in IGF-I concentrations between summer and winter ($p = 0.35$) (Table 2).

Age class differences

Serum ghrelin concentrations exhibited no significant differences among age classes ($p = 0.12$; Fig. 2A) and variance was also not different among age categories (HOV, $p = 0.12$, 1265.7 ± 281.8 pg/ml). Serum concentrations of GH were highly variable (HOV, $p = 0.03$, 8.0 ± 4.3 ng/ml) among age categories, and GH concentrations tended ($p = 0.07$; Fig2B) to be greater in calves (10.3 ± 1.8 ng/ml) and juveniles (10.1 ± 1.6 ng/ml) compared with adults (6.4 ± 1.0 ng/ml). There was no significant difference in IGF-I concentrations ($p = 0.28$; Fig. 2C) between age class and variability was similar (HOV, $p = 0.29$, 138.7 ± 51.2).

Discussion

While significant research has been conducted on metabolic hormones in domestic species (pigs [monogastric], cattle and sheep [ruminant]), very little information is available for exotic species (Govoni et al., 2011). Even more limited data are available for free-ranging ungulates and hindgut fermenters. Data presented here are critical for understanding the normal physiology of the endangered manatee as well as providing important observations for free-ranging hindgut fermenters.

The Florida manatee exhibited morphological, physiological, and hormonal differences among season. The Florida manatee may have similar seasonal patterns compared with temperate species, revealing that although they are in a warmer climate, moderate variation in photoperiod and temperature result in physiological response. Therefore, these data can provide a better overall understanding of the physiological response to photoperiod in species in sub-tropical environments to further monitor and protect their populations and ecosystems. Moreover, these data can offer another method of monitoring the Florida manatee health to help protect and conserve the species.

Seasonal differences

The Florida manatee experiences relatively minor seasonal variations in temperature, light, and food availability that may lead to physiological responses to cope with environmental pressures. They are a subtropical species that have a narrow thermoneutral zone and low metabolic rate influencing their need to migrate to warm water sites in order to seek refuge from cold temperatures (Reynolds and Odell, 1991; Reynolds and Wilcox, 1986). Like other marine mammals, manatees have blubber, multiple subcutaneous adipose layers that assist with their thermoregulatory needs during cold temperatures (Irvine, 1983). Unique to manatees are their thin alternating layers of blubber and paniculus muscle when compared with other marine mammals (Irvine, 1983). Concurrent with previous studies on domestic and wild species, manatees had reduced blubber thickness during summer compared with fall and winter; perhaps for advantageous competitive mating with greater adiposity in the fall and winter to maintain positive energy balance due to cold temperatures and the lack of food (Fuglei et al., 2004; Genin and Perret, 2000; Zinn et al., 1986). Additionally, increased blubber thickness in the fall may suggest adipose accumulation for thermoregulatory needs and energy demands for the winter season. Thicker blubber in the winter may be used to reduce thermoregulatory costs and provide energy for body maintenance when intake is reduced (Fuglei et al., 2004; Genin and Perret, 2000; Zinn et al., 1986).

Similar to sheep, ghrelin concentrations were consistent among seasons in the free-ranging Florida manatee (Iqbal et al., 2006). In a study by Iqbal et al. (2006), sheep were found to be unresponsive to the orexigenic properties of ghrelin because their gut is consistently full of food and not completely emptied between feedings (Sugino et al., 2002, 2004). Ghrelin is produced in the rumen and cecum of ruminant and hindgut fermenting species, respectively, versus the proximal region of the stomach in monogastric species (Date et al., 2000; Hayashida et al., 2001). The cecum houses microorganisms that function to digest cellulose in the process known as hindgut fermentation (Reynolds and Rommel, 1996). In sheep, ghrelin concentrations are consistently maintained across seasons, resulting in similar food intake throughout the year (Harrison et al., 2007). Even though sheep are foregut fermenting ruminants while, Florida manatee use hindgut fermentation, continuous gut fill is important for the efficiency of both digestive strategies. Although, studies have not been performed in manatees to determine where the abundance of ghrelin-producing cells are located it can be hypothesized that it may be in the cecum, similar to other hindgut fermenters (Hayashida et al., 2001). The gastrointestinal tract, including the cecum, of a manatee is not fully emptied between meals. Therefore ghrelin concentrations may not differ significantly among seasons because of the continuous presence of food in the lumen of the gastrointestinal tract (Iqbal et al., 2006; Reynolds and Rommel, 1996).

Growth hormone plays a role in tissue specific nutrient partitioning (Breier, 1999). Elevated GH concentrations promote lipolysis and inhibit lipogenesis (Breier, 1999; Fuglei et al., 2000). Greater GH concentrations in association with reduced blubber thickness in the summer suggests GH is stimulating IGF-I and protein accretion (Blumenthal et al., 2011; Breier, 1999). Whereas reduced GH in the winter and greater blubber thickness suggests blubber is being maintained or accumulated (Webster et al., 1996) instead of favoring adipose mobilization (lipolysis) for maintenance energy needs (Fuglei et al., 2000).

In previous ungulate studies, a seasonal response of IGF-I was evident. Concentrations were greater in the summer compared with the winter and were positively correlated with tissue accretion (Barenton et al., 1988; Webster et al., 1996). Manatees were predicted to have a similar response. As expected, IGF-I concentrations were greater in the summer; however, they did not differ
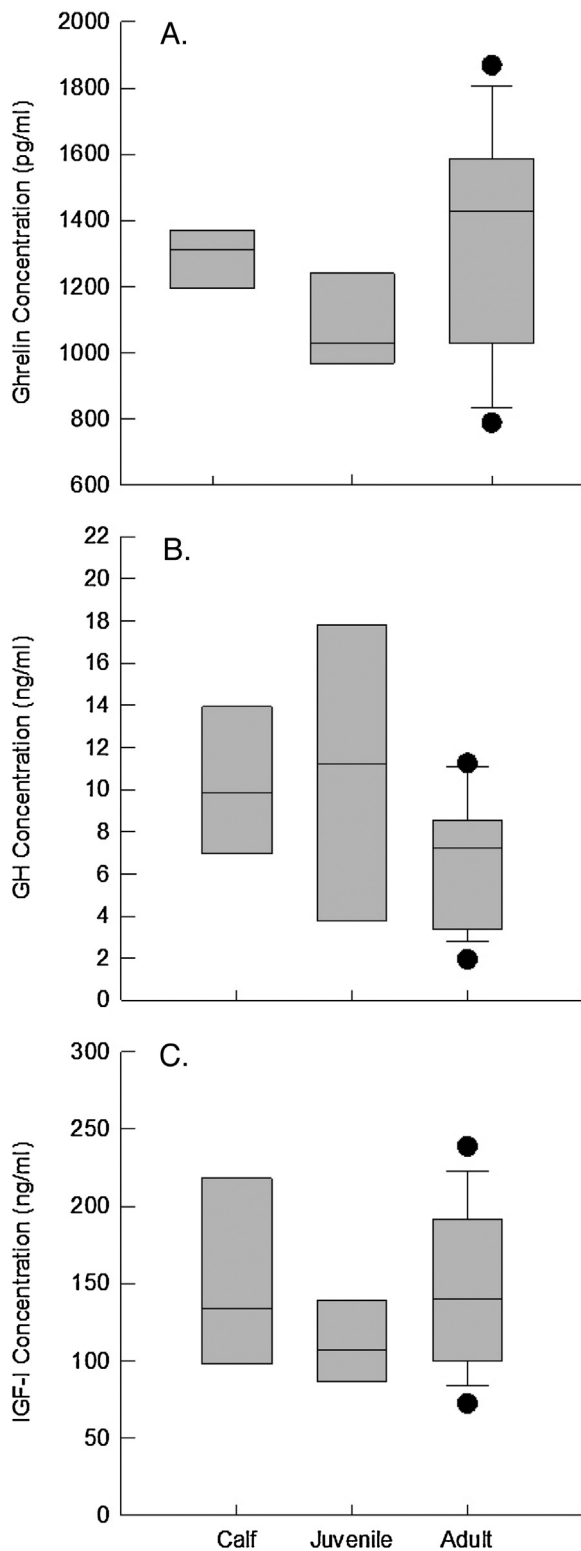
Fig. 2. (A–C) Average hormone concentrations among age classes (calves, juveniles, adults) during the winter from 2005–2010 in the free-ranging Florida manatee. Values presented as the mean ± SE in (A) ghrelin (pg/ml) (B) GH (ng/ml) and (C) IGF-I (ng/ml). Black dots are outliers beyond two standard deviations of the mean.

significantly from winter concentrations. In addition, IGF-I concentrations were reduced in the fall which was unexpected given the greater blubber depth in fall compared with summer suggesting adipose accumulation. Although, previous research suggests animals in temperate climates have greater IGF-I concentrations in

the fall compared with winter for adipose accumulation (Webster et al., 1996; Fuglei et al., 2004), perhaps low concentrations of IGF-I in the Florida manatee are sufficient to increase and maintain blubber. Alternatively, these reduced IGF-I concentrations in the fall may suggest reduced intake as manatee are traveling to warm water refuge for the winter (Reynolds and Rommel, 1996).

Insulin-like growth factor-I stimulates protein accretion and works with GH to facilitate adipose and lean tissue accretion (Gautsch et al., 1998; Hornick et al., 2000). Greater concentrations of GH and IGF-I are positively correlated with growth rate and food intake in red deer during the spring season and are thought to produce rapid lean tissue accretion (Adam et al., 1996; Suttie et al., 1989; Webster et al., 1996). Alternatively, reduced GH concentrations in association with greater IGF-I concentrations during the fall and winter suggest the facilitation of lipogenesis (Rosenfeld and Hwa, 2009). Manatees had elevated GH and IGF-I concentrations in conjunction with reduced blubber, suggesting lean tissue accretion. This may be beneficial to manatees due to the peak in mating activity and reproductive demands during the summer and fall seasons (Hartman, 1979; Reep and Bonde, 2006; Rosenfeld and Hwa, 2009). Although GH concentrations were not significantly different in fall compared with summer and winter, greater GH from summer to winter in conjunction with reduced IGF-I and greater blubber thickness suggest manatees may be accumulating adipose for the winter as seen in other species like the arctic fox and red deer (Fuglei et al., 2004; Webster et al., 1996, 1999). Greater IGF-I, reduced GH, in conjunction with greater blubber thickness during the winter may suggest maintenance of blubber stores to meet the thermoregulatory needs due to colder temperatures. Changing hormone concentrations in association with blubber thickness among summer, fall, and winter seasons suggest seasonal changes in nutrient priorities in the Florida manatee follow a similar pattern.

Age-class differences

While little is known about ghrelin during post-natal development, the role of GH and IGF-I have been well studied in domestic species growth and development. Most species exhibit an age related decline in GH while IGF-I generally increases with age (Comeau et al., 2008; Govoni et al., 2003; Harrell et al., 1999). Importantly, both GH and IGF-I are elevated during periods of rapid growth such as the early post-natal period as well as puberty (Govoni et al., 2003; Harrell et al., 1999). Manatee were hypothesized to follow a similar pattern; however, in this study not all hormones exhibited the typically mammalian developmental pattern.

Ghrelin is highly conserved across species (Kojima et al., 1999), and is found primarily in the stomach (Ariyasu et al., 2001; Date et al., 2000). Although ghrelin is known to stimulate food intake, adiposity, and the release of GH (Korbonits et al., 2004; Nakazato et al., 2001; Tschop et al., 2000), the role ghrelin plays in development is still unclear (Akimoto et al., 2011; Chanoine et al., 2006). Although there are limited studies on the ontogeny of ghrelin, it was hypothesized that ghrelin concentrations may be constant between age classes species to stimulate appetite maintain a positive nutritional status. In the present study, ghrelin concentrations exhibited no differences among age classes in the free-ranging Florida manatee. Manatee are hindgut fermenters with food continuously in their gastrointestinal tract which may be contributing to consistent ghrelin concentrations between age classes (Iqbal et al., 2006; Reynolds and Rommel, 1996). This may also suggest food intake is relatively constant with maturation in manatees.

In most mammalian species including humans, cattle, and sheep, GH generally declines with age, with increased secretion during the perinatal and pubescent stages associated with periods of increased growth rate (Albertsson-Wikland et al., 1994;

Anderson et al., 1988; Blackman et al., 1994; Martha et al., 1992; Schwarz et al., 1992). Similar to these species, the free-ranging Florida manatee exhibited reduced GH concentrations in adults compared with calves and juveniles. Manatee calf growth rate is high the first few years of life during and after nursing. Calves range from 91 to 137 cm in length at birth and can reach 152 to 183 cm within the first two years of life (Odell, 1982). As seen in previous research, greater GH concentrations in calves and juveniles suggest a greater growth rate (Anderson et al., 1988; Albertsson-Wikland et al., 1994; Blackman et al., 1994; Martha et al., 1992; Schwarz et al., 1992). In addition, the juvenile age class exhibited a wide range of GH concentrations demonstrating high variability. The juveniles used in this study may include prepubescent and pubescent individuals increasing the likelihood that some animals may be experiencing rapid growth which may also explain the wide range of GH concentrations; increased GH concentrations in individuals experiencing puberty and therefore, growth, may contribute to the high variability observed in this group (Govoni et al., 2003; Marmontel, 1993; Schwarz et al., 1992; Zinn et al., 1989).

Serum IGF-I concentrations are known to increase significantly during the first few years of life and then begin to decrease with age eventually plateauing after puberty (Blackman et al., 1994; Comeau et al., 2008; Gatford et al., 1996; Govoni et al., 2003; Harrell et al., 1999; Schwarz et al., 1992). Surprisingly, there were no differences in IGF-I concentrations among age classes in the free-ranging Florida manatee. Previous studies found human, cattle, pig, and lamb IGF-I concentrations were initially elevated while nursing, reduced post-weaning, followed by an increase as animals approach puberty, and plateaued when individuals reached puberty (Blackman et al., 1994; Gatford et al., 1996; Govoni et al., 2003; Harrell et al., 1999; Schwarz et al., 1992). It is likely that the manatee calves used for the current study were no longer in their initial perinatal stage when captured; neonate calves are not often captured during health assessments due to the stress placed on the calf and its mother. In addition, the manatee calves used for the present study may no longer have been nursing and therefore, IGF-I concentrations were consistent among the age classes. Younger nursing calves may exhibit increased IGF-I concentrations similar to other mammalian species.

During normal physiological conditions, GH stimulates the release of IGF-I. Together the hormones are associated with growth rate and protein metabolism. Increased GH and IGF-I concentrations in the perinatal and pubescent stages of life in domestic species promote increased growth rate (Albertsson-Wikland et al., 1994; Anderson et al., 1988; Blackman et al., 1994; Martha et al., 1992; Schwarz et al., 1992). In the current study, greater GH concentrations and consistent IGF-I and ghrelin concentrations in calf and juvenile manatees suggest greater growth rate. Although, IGF-I and ghrelin concentrations were consistent with the adult age class, greater GH may stimulate growth. Whereas reduced GH in adults and the consistency in IGF-I and ghrelin concentrations may no longer stimulate growth rate, but rather changes in body composition. The differences in GH between age classes suggest that there are age-related changes occurring; however, future research needs to be conducted to further understand how these hormones specifically impact growth rate in the Florida manatee.

Conclusions

Based on previous seasonal research conducted in high latitude mammals, manatees shared interesting seasonal patterns with temperate species. However, age related changes in metabolic hormones did not follow the typical mammalian pattern. Ghrelin, GH, and IGF-I profiles in conjunction with body composition changes between seasons indicated seasonal adjustments in man-

atee tissue-specific nutrient partitioning. Ghrelin concentrations were similar to domestic ruminant species remaining consistent through changes in season. These data suggest manatees are prioritizing lean tissue accretion during the summer for potential mating and reproductive demands, whereas, manatees may be prioritizing adiposity in the fall and winter for energy maintenance and thermoregulatory needs.

The Florida manatee is an endangered species protected by the Marine Mammal Protection Act (MMPA, 1972) and the Endangered Species Act (ESA, 1973). While ecological and population studies have been conducted in this species, limited research has been completed on the physiology of manatee. Understanding the normal physiological mechanisms that contribute to manatee growth, development, and changes in nutrient intake are important considerations for understanding how manatee will cope with changing environmental conditions. The Florida manatee has a low metabolic rate compared to other mammals that reduces their ability to maintain their core body temperature during the winter season which contributes to their susceptibility to cold and ultimately induces a physiological reaction contributing to clinical symptoms (i.e., emaciation, fat store depletion) and disease referred to as cold-stress syndrome (Bossart et al., 2002; Irvine, 1983). Determining the normal pattern of seasonality in the Florida manatee may provide a better understanding for managers to develop successful methods to determine population health status and further the survival of the species.

References

Adam, C.L., Kyle, C.E., Young, P., 1996. Seasonal patterns of growth, voluntary food intake and plasma concentrations of prolactin insulin-like growth factor-I, LH and gonadal steroids in male and female pre-pubertal red deer (Cervus elaphus) reared in either natural photoperiod or constant daylength. Anim. Sci. 62, 605–614.

Akimoto, Y., Kanai, S., Minoru, O., Akimoto, S., Uematsu, H., Miyasaka, K., 2011. Age-associated reduction of stimulator effect of ghrelin on food intake in mice. Arch. Gerontol. Geriatr., http://dx.doi.org/10.1016/j.archger.2011.09.007.

Albertsson-Wikland, K., Roseberg, S., Kalberg, J., Groth, T., 1994. Analysis of 24-hour growth hormone profiles in healthy boys and girls of normal stature: relation to puberty. J. Clin. Endocrinol. Metab. 78, 1195–1201.

Anderson, P.T., Bergen, W.G., Merkel, R.A., Enright, W.J., Zinn, S.A., Refsal, K.R., Hawkins, D.R., 1988. The relationship between composition of gain and circulating hormones in growing beef bulls fed three dietary crude protein levels. J. Anim. Sci. 66, 3059.

Ariyasu, H., Takaya, K., Tagami, T., Ogawa, Y., Hosoda, K., Akamizu, T., Suda, M., Koh, T., Natsui, K., Toyooka, S., Shirakami, G., Usui, T., Shimatsu, A., Doi, K., Hosoda, H., Kojima, M., Kangawa, K., Nakao, K., 2001. Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like immunoreactivity levels in humans. J. Clin. Endocrinol. Metab. 86, 4753–4758.

Barenton, B., Ravault, J.P., Chabanet, C., Daveau, A., Pelletier, J., Ortavant, R., 1988. Photoperiodic control of growth hormone secretion and body weight in rams. Domest. Anim. Endocrinol. 5, 247–255.

Bergan, H.E., Kittilson, J.D., Sheridan, M.A., 2015. Nutritional state modulates growth hormone-stimulated lipolysis. Gen. Comp. Endocrinol. 218, 1–9, http://dx.doi.org/10.1016/j.ygcen.2015.04.017.

Blackman, M.R., Vittone, J., Corpas, E., 1994. Growth hormone releasing hormone: effects in healthy elderly men. Growth Hormone Secretagogues, vol. 23. Int. Serono Symp., USA.

Blumenthal, S., Morgan-Boyd, R., Nelson, R., Garshelis, D.L., Turyk, M.E., Unterman, T., 2011. Seasonal regulation of the growth hormone-insulin-like growth factor-I axis in the American black bear (*Ursus americanus*). Am. J. Physiol. Endocrinol. Metab. 301, E628–E636, http://dx.doi.org/10.1152/ajpendo.00082.2011.

Bonde, R.K., Garrett, A., Belanger, M., Askin, N., Tan, L., Wittnich, C., 2012. Biomedical health assessments of the Florida manatee in Crystal River—providing opportunites for training during capture, handling, and processing of this endangered aquatic mammal. J. Mar. Anim. Ecol. 5, 17–28.

Bossart, G.D., Reidarson, T.H., Dierauf, L.A., Duffield, D.A., 2001. Clinical pathology. In: Dierauf, L.A., Gulland, F.M. (Eds.), CRC Handbook of Marine Mammal Medicine. CRC Press, Boca Raton, Florida, pp. 383–390.

Bossart, G.D., Meisner, R., Rommel, S.A., Ghim, S., Jenson, A.B., 2002. Pathological features of the Florida manatee cold stress syndrome. Aquat. Mamm. 29, 9–17.

Breier, B.H., 1999. Regulation of protein and energy metabolism by the somatotropic axis. Domest. Anim. Endocrinol. 17, 209–218.

Cammisotto, P.G., Levy, E., Bukowiecki, L.J., Bendayan, M., 2010. Cross-talk between adipose and gastric leptins for the control of food intake and energy metabolism. Prog. Histochem. Cytochem. 45, 143–200.

Castaneda, T.R., Tong, J., Datta, R., Culler, M., Tschop, M.H., 2010. Ghrelin in the regulation of body weight and metabolism. Front. Neuroendocr. 31 (1), 44–66.

Chanoine, J.P., Wong, A.C., Barrios, V., 2006. Obestatin, acylated and total ghrelin concentrations in the perinatal rat pancreas. Horm. Res. 66, 81–88.

Comeau, G.A., Richmond, J.P., McCusker Shipley, S.L., Koutsos, E., Zinn, S.A., 2008. Developmental trends in components of the somatotropic axis of mule deer (*Odocoileus hemionus*) fawns fed three different herbivore diets. Comp. Nutr. Soc. Proc. 7, 55–59.

Cummings, D.E., Purnell, J.Q., Frayo, R.S., Schmidova, K., Wisse, B.E., Weigle, D.S., 2001. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. Diabetes 50, 1714–1719.

Date, Y., Kojima, Y., Hosoda, H., Sawaguchi, A., Mondal, M.S., Suganuma, T., 2000. Ghrelin a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. Endocrinology 141, 4255–4261.

Endangered Species Act, 1973, United States Congress.

Etherton, T.D., 1991. The role of insulin-like growth factors (IGF) and the IGF-binding proteins in growth and metabolism. In: Pearson, A.M., Dutson, T.R. (Eds.), Growth & Regulation in Farm Animals, Advances in Meat Research, vol. 7. Elsevier Science Publishers Ltd., London, UK, pp. 343–372.

Florant, G.L., Healy, J.E., 2012. The regulation of food intake in mammalian hibernators: a review. J. Comp. Physiol. B 182, 451–467.

Fuglei, E.M., Aanestad, M., Berg, J.P., 2000. Hormones and metabolites of arctic foxes *Alopex lagopus* in response to season: starvation and re-feeding. Comp. Biochem. Physiol. A: Mol. Intergr. Physiol. 126, 287–294.

Fuglei, E., Mustonen, A.M., Nieminen, P., 2004. Effects of season, food deprivation and re-feeding on leptin ghrelin and growth hormone in arctic foxes (*Alopex lagopus*) on Svalbard, Norway. J. Comp. Physiol. 174, 157–162.

Gallivan, G.J., Best, R.C., Kanwisher, J.W., 1983. Temperature regulation in the Amazonian manatee *Trichechus inunguis*. Physiol. Zool. 56, 255–262.

Gatford, K.L., Fletcher, T.P., Clarke, I.J., Owens, P.C., Quinn, K.J., Walton, P.E., Grant, P.A., Hosking, B.J., Egan, A.R., Ponnampalam, E.N., 1996. Sexual dimorphism of circulating somatotropin, insulin-like growth factor I and II, insulin-like growth factor binding proteins, and insulin: relationships to growth rate and carcass characteristics in growing lambs. J. Anim. Sci. 74, 1314–1325.

Gautsch, T.A., Kandl, S.M., Donovan, S.M., Layman, D.K., 1998. Response of the IGF-I system to prolonged under nutrition and its involvement in somatic and skeletal muscle growth retardation in rats. Growth Dev. Aging 62 (1), 13–25.

Genin, F., Perret, M., 2000. Photoperiod-induced changes in energy balance in gray mouse lemurs. Physiol. Behav. 71, 315–321.

Gerrard, D.E., Grant, A.L., 2006. Principles of Animal Growth & Development. Kendall/Hunt Publishing Co., Dubuque, IA, pp. 189–192.

Govoni, K.E., Hoagland, T.A., Zinn, S.A., 2003. The ontogeny of the somatotropic axis in male and female Hereford calves from birth to one year of age. J. Anim. Sci. 81, 2811–2817.

Govoni, K.E., Goodman, D., Maclure, R.M., Penfold, L.M., Zinn, S.A., 2011. Serum concentrations of insulin-like growth factor-I and insulin-like growth factor binding protein-2 and -3 in eight hoofstock species. Zoo Biol. 30, 275–284.

Harrell, R.J., Thomas, M.J., Boyd, R.D., Czerwinski, S.M., Steele, N.C., Bauman, D.E., 1999. Ontogenic maturation of the somatotropin/insulin-like growth factor axis. J. Anim. Sci. 77, 2934–2941.

Harrison, J.L., Miller, D.W., Findlay, P.A., Adam, C.L., 2007. Photoperiod influences the central effects of ghrelin on food intake, GH and LH secretion in sheep. Neuroendocrinology 87, 182–192.

Hartman, D.S., 1979. Ecology and Behavior of the Manatee (*Trichechus manatus*) in Florida. American Society of Mammals Special Publication Number 5, 153 pages.

Hayashida, T., Murakami, K., Mogi, K., Nishihara, M., Nakazato, M., Mondal, M., Horii, Y., Kojima, M., Kangawa, K., Murakami, N., 2001. Ghrelin in domestic animals: distribution in stomach and its possible role. Domest. Anim. Endocrinol. 21, 17–24.

Hornick, J.L., Van Eenaeme, C., Gérard, O., Dufrasne, I., Istasse, L., 2000. Mechanisms of reduced and compensatory growth. Domest. Anim. Endocrinol. 19, 121–132.

Iqbal, J., Kurose, Y., Canny, B., Clarke, I.J., 2006. Effects of central infusion of ghrelin on food intake and plasma levels of growth hormone, luteinizing hormone, prolactin, and cortisol secretion in sheep. Endocrinology 147, 510–519.

Irvine, A.B., 1983. Manatee metabolism and its influence on distribution in Florida. Biol. Conserv. 25, 315–334.

Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H., Kangawa, K., 1999. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 402, 656–660.

Korbonits, M., Goldstone, A.P., Gueorguiey, M., Grossman, A.B., 2004. Ghrelin—a hormone with multiple functions. Front. Neuroendocr. 25, 27–68.

Lawrence, T.L.J., Fowler, V.R., 1997. Hormonal, genetic and immunological influences of growth. In: Growth of Farm Animals. CAB International, Wallingford Oxon, U.K, pp. 104–110.

Marine Mammal Protection Act, 1972. United States Congress.

Marmontel, M., 1993. Age determination and population biology of the Florida manatee *Trichechus manatus latirostris*. In: Ph.D. Dissertation. University of Florida, Gainsville, Florida, 408 pp.

Martha, P.M., Gorman, K.M., Blizzard, R.M., Rogol, A.D., Veldhuis, J.D., 1992. Endogenous growth hormone secretion and clearance rates in normal boys, as determined by deconvolution analysis: relationship to age, pubertal status, and body mass. J. Clin. Endocrinol. Metab. 74, 336–344.

Nakazato, M., Murakami, N., Date, Y., Kojima, M., Matsuo, H., Kangawa, K., Matsukura, S., 2001. A role for ghrelin in the central regulation of feeding. Nature 409, 194–198.

Nunes, S., CDT, H.A., Garrett, P.J., Mueke, E., Smale, L., Holekamp, K.E., 1998. Body fat and time of year interact to mediate dispersal behavior in ground squirrels. Anim Behav 55, 605–614.

Odell, D.K., 1982. The West Indian Manatee Trichechus Manatus Linnaeus. Wild Mammals of North America, 1184. Johns Hopkins University Press Baltimore, pp. 828–837.

Prestrud, P., Nilssen, K., 1992. Fat deposition and seasonal variation in body composition of arctic foxes in Svalbard. J. Wildl. Manag., 221–233.

Reep, R.L., Bonde, R.K., 2006. The Florida Manatee: Biology and Conservation. University Press of Florida, 189 pages.

Renaville, R., Hammadi, M., Portetelle, D., 2002. Role of the somatotropic axis in the mammalian metabolism. Domest. Anim. Endocrinol. 23, 351–360.

Reynolds III., J.E., 2000. Possible Locations for Long-term, Warm-water Refugia for Manatees in Florida: Alternatives to Power Plants. Report to Florida Power and Light Company.

Reynolds III., J.E., Odell, D.K., 1991. Manatees and Dugongs. Facts on File, Inc., New York, pp. 192.

Reynolds III., J.E., Rommel, S.A., 1996. Structure and function of the gastrointestinal tract of the Florida manatee: *Trichechus manatus latirostris*. Anat. Rec. 245, 539–558.

Reynolds III., J.E., Wilcox, J.R., 1986. Distribution and abundance of the West Indian manatee *Trichechus manatus* around selected Florida power plants following winter cold front: 1984–85. Biol. Conserv. 38, 103–113.

Richmond, J.P., Zinn, S.A., 2009. Validation of heterologous radioimmunoassays (RIA) for growth hormone (GH) and insulin-like growth factor (IGF)-I in phocid, otariid, and cetacean species. Aquat. Mamm. 35 (1), 19–31.

Rosenfeld, R.G., Hwa, V., 2009. The growth hormone cascade and its role in mammalian growth. Horm. Res. Pediatr. 71, 36–40.

Salbe, A.D., Tschop, M.H.I., Parigi, A., Venti, C.A., Tataranni, P.A., 2004. Negative relationship between fasting plasma ghrelin concentrations and ad libitum food intake. J. Clin. Endocrinol. Metab. 89, 2951–2956.

Schwarz, F.J., Ropke, R., Schams, D., Kirchgessner, M., 1992. Effects of sex and growth on plasma concentrations of growth hormone, insulin-like growth factor-I and insulin in fattening Simmental cattle. J. Anim. Physiol. 68, 263–271.

Stamper, M.A., Bonde, R.K., 2012. Health assessment of captive and Wild-caught West Indian manatees (*Trichechus manatus*). In: Hines, Reynolds, Aragones, Mignucci-Giannoni, Marmontel (Eds.), Sirenian Conservation: Issues and Strategies in Developing Countries. University Press of Florida, Gainesville, Florida (Chapter 16. Pages 139–147, 326 pp.

Sugino, T., Hasegawa, Y., Kikkawa, Y., Yamaura, J., Yamagishi, M., Kurose, Y., Kojima, M., Kangawa, K., Terashima, Y., 2002. A transient ghrelin surge occurs just before feeding in a scheduled meal-fed sheep. Biochem. Biophys. Res. Commun. 295, 255–260.

Sugino, T., Hasegawa, Y., Kurose, Y., Kojima, M., Kangawa, K., Terashima, Y., 2004. Effects of ghrelin on food intake and neuroendocrine function in sheep. Anim. Reprod. Sci. 82, 183–194.

Suttie, J.M., Fennessy, P.F., Corson, I.D., Laas, F.J., Crosbie, S.F., Butler, J.H., Gluckman, P.D., 1989. Pulsatile growth hormone, insulin-like growth factors and antler development in red deer (*Cervus elaphus scoticus*) stags. J. Endocrinol. 12, 351–360.

Tschop, M., Smiley, D.L., Heiman, M.L., 2000. Ghrelin induces adiposity in rodents. Nature 407, 908–913.

Vijayakumar, A., Novosyadlyy, R., Wu, Y., Yakar, A., LeRoith, D., 2010. Biological effects of growth hormone on carbohydrate and lipid metabolism. Growth Horm. IGF Res. 20, 1–7.

Ward-Geiger, L.I., 1997. Blubber depth and body condition indices in the Florida manatee. (*Trichechus manatus latirostris*). In: MS Thesis. University of South Florida, St. Petersburg, Florida, USA, pp. 119.

Webster, J.R., Corson, I.D., Littlejohn, R.P., Stuart, S.K., Suttie, J.M., 1996. Effects of season and nutrition on growth hormone and insulin-like growth factor-I in male red deer. Endocrinology 137, 698.

Webster, J.R., Corson, I.D., Littlejohn, R.P., Stuart, S.K., Suttie, J.M., 1999. Effects of photoperiod on the cessation of growth during autumn in male red deer and growth hormone and insulin-like growth factor-I secretion. Gen. Comp. Endocrinol. 113, 464–477.

Wong, A.W., Bonde, R.K., Siegal-Willott, J., Stamper, M.A., Collee, J., Powell, J.A., Reid, P.R., Deutsch, C.J., Harr, K.E., 2012. Monitoring oral temperature, heart rate, and respiration rate of West Indian manatees (*Trichechus manatus*) during capture and handling in the field. Aquat. Mamm. 38, 1–16.

Zhang, L., Zhang, H., Zhu, W., Li, X., Wang, Z., 2012. Energy metabolism, thermogenesis and body mass regulation in tree shrews (*Tupaia belangeri*) during subsequent cold and warm acclimation. Comp. Biochem. Physiol. A 162, 437–442.

Zhu, W., Zhang, H., Wang, Z., 2012. Seasonal changes in body mass and thermogenesis in tree shrews (*Tupaia belangeri*): the roles of photoperiod and cold. J. Therm. Biol. 37, 479–484.

Zinn, S.A., Purchas, R.W., Chapin, L.T., Petitclerc, D., Merkel, R.A., Bergen, W.G., 1986. Effects of photoperiod on growth, carcass composition, prolactin, growth hormone and cortisol in prepubertal and postpubertal Holstein heifers. J. Anim. Sci. 63, 1804–1815.

Zinn, S.A., Chapin, L.T., Enright, W.J., Tucker, H.A., 1989. Growth, carcass composition and serum hormone responses to photoperiod and ovariectomy in heifers. Anim. Prod. 49, 365–373.