Integrative and Comparative Biology

Integrative and Comparative Biology, volume 54, number 3, pp. 463–483
doi:10.1093/icb/icu001

Society for Integrative and Comparative Biology

THEME AND VARIATIONS: HETEROTHERMY IN MAMMALS

Endocrine Regulation of Bone and Energy Metabolism in Hibernating Mammals

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Synopsis Precise coordination among organs is required to maintain homeostasis throughout hibernation. This is particularly true in balancing bone remodeling processes (bone formation and resorption) in hibernators experiencing nutritional deprivation and extreme physical inactivity, two factors normally leading to pronounced bone loss in non-hibernating mammals. In recent years, important relationships between bone, fat, reproductive, and brain tissues have come to light. These systems share interconnected regulatory mechanisms of energy metabolism that potentially protect the skeleton during hibernation. This review focuses on the endocrine and neuroendocrine regulation of bone/fat/energy metabolism in hibernators. Hibernators appear to have unique mechanisms that protect musculoskeletal tissues while catabolizing their abundant stores of fat. Furthermore, the bone remodeling processes that normally cause disuse-induced bone loss in non-hibernators are compared to bone remodeling processes in hibernators, and possible adaptations of the bone signaling pathways that protect the skeleton during hibernation are discussed. Understanding the biological mechanisms that allow hibernators to survive the prolonged disuse and fasting associated with extreme environmental challenges will provide critical information regarding the limit of convergence in mammalian systems and of skeletal plasticity, and may contribute valuable insight into the etiology and treatment of human diseases.

Introduction

Excessive accumulation of fat via hyperphagia prior to hibernation and reduced metabolic rate during hibernation allows mammalian hibernators (e.g., *Ursus* and *Marmota*) to survive periods of prolonged famine and extreme ambient temperatures (T_a) with little, if any, adverse effects to tissues and organs. This is likely accomplished in part by neural, hormonal, and metabolic changes that promote energy conservation and tissue and organ maintenance. Precise coordination between many organs is required to maintain homeostasis for the duration of the hibernation season. In recent years, important relationships between bone, fat, reproductive, and brain tissues in mammals have come to light; all four systems share interconnected regulatory mechanisms of energy metabolism. Understanding the biological mechanisms by which hibernators can survive the prolonged disuse and fasting associated with extreme environmental challenges will provide critical information regarding the limit of convergence in mammalian systems and of skeletal plasticity, and may contribute valuable insight into human disease.

Several possible strategies are available to mammals for surviving prolonged periods of harsh environmental conditions with limited or no food: migration to food-rich environments, storage of food in caches, storage of energy in the form of fat, reduction in expenditure of metabolic energy, or a combination of these strategies. Hibernating animals have the ability to lower endogenous energy demands by lowering body temperature (T_b) and metabolism for multiple days (torpor bouts) (Carey et al. 2003), and thus are able to survive long periods of fasting (i.e., during winter) without changing their environmental location. Food-storing hibernators (e.g., *Glis*) are more active and continue to eat food throughout the winter, unlike fat-storing hibernators. Excessive accumulation of fat by small hibernating rodents (<400 g) would limit their mobility and increase the risk of predation, but with food-storage strategies they are capable of collecting large hoards of food exceeding

Advanced Access publication February 19, 2014

their body size (Humphries et al. 2003). Fat-storing hibernators, such as marmots (*Marmota flaviventris*) and some ground squirrels (*Callospermophilus*), on the other hand, must forage enough to fatten prior to winter (maximum fat reserves are 40–50% body mass). In these animals, large amounts of triglycerides are deposited in white adipose tissue during preparation for hibernation (Dark 2005). During torpor, fat-storing hibernators switch from carbohydrate catabolism to lipid-based metabolism (Storey and Storey 2010). Stores of fat and oxidation of lipids are used almost exclusively to maintain cellular processes and survive the winter when food is absent (Galster and Morrison 1975). Indeed, the metabolic switch to lipid hydrolysis characteristic of animals that are capable of torpor has been extensively studied and is quite complex (Carey et al. 2003; Melvin and Andrews 2009). Thus, hibernation is essentially the conservation of energy during seasonal periods of scarcity of food (Melvin and Andrews 2009). As such, hibernating animals experience dramatic reduction in their basal metabolic rate (as low as 2–5% of summer levels) by going from a homeothermic (~37°C) state to one of heterothermy (torpor), during which T_b can vary between euthermia and near T_a (Florant and Heller 1977; Buck and Barnes 2000; Geiser 2004, 2013; Storey and Storey 2010). Such fluctuations are indicative of extreme changes in the regulation of organ function and cellular processes.

The skeleton and homeostasis of calcium during hibernation

Disuse in non-hibernators, including humans, causes unbalanced bone remodeling and bone loss (Zerwekh et al. 1998; Shackelford et al. 2004; Li et al. 2005). Imbalances in bone remodeling typically lead to hypercalcemia and increased excretion of calcium. However, hibernating bears, woodchucks (*Marmota monax*), and marmots maintain balanced bone remodeling and are capable of limiting loss of muscle and skeletal mass (Floyd et al. 1990; Harlow et al. 2001; Pardy et al. 2004; Lohuis et al. 2007; McGee et al. 2008; McGee-Lawrence et al. 2009a, 2009b; Doherty et al. 2012; Doherty 2013). In fact, grizzly bears (*Ursus arctos horribilis*) reduce cortical bone remodeling during hibernation to 25% of summer levels (McGee et al. 2008), which is similar to the overall reduction in metabolism in American black bears (*Ursus americanus*) (Tøien et al. 2011). Balanced bone remodeling during hibernation is likely driven by the need to maintain eucalcemia during seasonal anorexia and anuria. Indeed, calcium regulatory hormones (e.g., parathyroid hormone [PTH]) may tightly control calcium concentrations in the serum during hibernation (Donahue et al. 2006a). Furthermore, the consequences to the skeleton from lack of an adequate diet suggest that hibernating animals would experience significant bone loss from nutritional deprivation in addition to the loss from physical inactivity. Non-hibernating mammals, in contrast, experience severe negative consequences with nutritional deficiency. In humans, "anorexia nervosa" increases resorption of bone and decreases bone formation to eventually result in osteoporosis (Powers 1999). Thus, it is believed that reduced bone turnover contributes to the conservation of energy during hibernation and balanced bone resorption/formation prevents osteoporosis.

Excessive seasonal accumulation of fat by hibernators has several implications for the skeletal health of these animals. There is a high degree of cross-talk among adipokines, sex hormones, and energy/bone metabolism as evidenced by the involvement of endocrine factors (e.g., leptin) in multiple systems. Hormones derived from fat negatively influence skeletal regulation by favoring bone resorption relative to bone formation in mice (Ducy et al. 2000). Thus, the processes controlling and balancing the body systems involved in the metabolism of fat and bone are complex (Fig. 1). Indeed, the metabolic syndrome characteristic of high abdominal adiposity, fatty diet, and sedentary lifestyle increases the risk of developing severe cardiovascular disease, type II diabetes, hypertension, and a number of other metabolic disorders (Cornier et al. 2008). Furthermore, some bone fractures may be attributed to obesity (Mathey et al. 2002; Goulding et al. 2005; Hsu et al. 2006; Cao et al. 2010; Patsch et al. 2011).

The purpose of this article is to review the metabolism of energy as regulated by the interactions of fat with the skeleton through endocrine and neuroendocrine pathways and to discuss these mechanisms as they pertain to hibernators (Tables 1–4). Data are grouped by body system in Tables 1–4, and some examples of each metabolic factor are given in the tables and text. The values in Tables 1–4 were estimated from graphs and charts when numerical values were not reported. The hormones listed in the tables are not exhaustive of the extensive literature on these topics, and individual differences among studies should be kept in mind. Furthermore, studies of serum are preferentially presented for maintaining consistency of the data, and for that reason tissue homogenates or histological data typically are not reported here. An additional confounding factor potentially contributing to variation in data between studies is likely because of

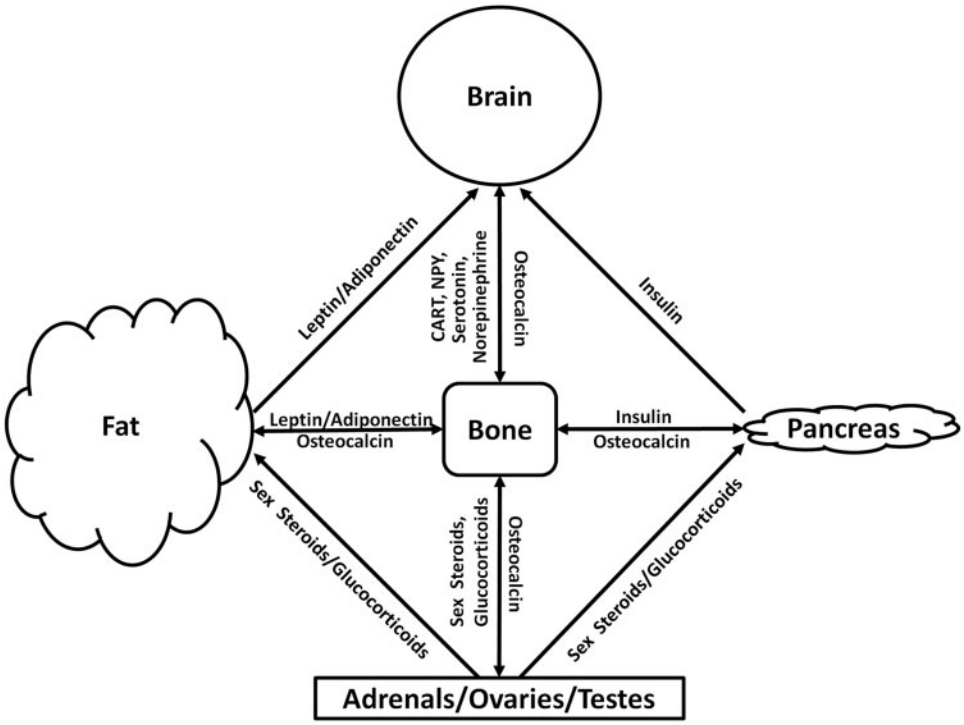

Fig. 1 The interrelationships of bone. Energy homeostasis involves regulation of bone by adipose tissue and the pancreas. The gonads and brain also contribute significantly to bone metabolism. There is considerable cross-talk among systems, including the endocrine signaling of bone in fat-deposition, reproduction, and neuroprotective functions.

different time points of sample collection (i.e., seasonal sample collections are not necessarily comparable). It is becoming increasingly apparent that seasonal groupings (such as pre-hibernation, hibernation, and post-hibernation) do not necessarily capture physiological changes in short-term transitional stages (e.g., the transition periods from pre-hibernation to hibernation and from hibernation to post-hibernation). Therefore, researchers need to define these seasonal categories in a consistent way. With these caveats in mind, this article reviews the physiological processes that may impact bone in hibernators.

Regulators of fat, bone, and energy metabolism in hibernating mammals

Leptin

Leptin plays a role in energy metabolism by controlling appetite and body mass. Long before its genetic identification, leptin was identified as the satiety factor because of its negative effects on long-term intake of food and maintenance of a lean-mass phenotype (Coleman 1978). Mice that are deficient in leptin (ob/ob) and leptin receptor (db/db) are obese, hyperphagic, and experience high serum levels of insulin and glucose (Coleman 1978; Zhang et al. 1994; Tartaglia et al. 1995). Leptin is produced by white and brown adipocytes and circulates in plasma to signal, primarily to the hypothalamus, the amount of stored body fat (Maffei et al. 1995).

Diets high in fat not only result in obesity and high levels of circulating leptin but also an inability to decrease the intake of food (Frederich et al. 1995). This resistance to the effects of leptin, or hyperleptinemia, is attributed to the inability of leptin to induce the signaling cascades responsible for inhibiting appetite. Leptin is not transported across the blood–brain barrier (BBB) during induced peripheral hyperleptinemia (Banks et al. 1996, 2006; Caro et al. 1996; Halaas et al. 1997; Van Heek et al. 1997; Tu et al. 2008). The short, soluble leptin receptor (ObR) normally transports leptin across the BBB (Lee et al. 1996; Kastin et al. 1999; Hileman et al. 2002; Tu et al. 2008) and stimulates various hypothalamic nuclei to initiate multiple signaling cascades (Lee et al. 1996). This includes regulating hypothalamic neuropeptides important in feeding and maintenance of energy. Low levels of circulating leptin promote the production of neuropeptide-Y (NPY) and agouti-related protein (AGRP) which increase the intake of food and decrease energy expenditure (Stanley et al. 1986; Billington et al. 1991; Shutter et al. 1997; Rossi et al. 1998). Alternatively, high

Table 1 Summary of some of the reported metabolic factors influencing bone metabolism in hibernators[a]

		Seasonal concentrations					
			Winter (hibernation)				
Hormone(s)	Species	Fall (pre-hibernation)	Early hibernation	Late hibernation	Spring (post-hibernation)	Summer (active)	References
Leptin	American Black Bear (*U. americanus*)	4.0 ng/ml	3.66–3.8 ng/ml		2.08–3.1 ng/ml		Donahue et al. (2006a, 2006b) and Seger (2008)
	Woodchuck (*M. monax*)	1.11 ng/ml	0.035–0.672 ng/ml		0.888–0.1 ng/ml	0.48–1.0 ng/ml	Doherty (2013) and Concannon et al. (2001)
	Marmot (*M. flaviventris*)	9.7 ng/ml	10.7 ng/ml	4.3 ng/ml		3.2 ng/ml	Florant et al. (2004)
	Golden-mantled Ground Squirrel (*C. lateralis*)	12.7 ng/ml	6.25 ng/ml	2.7 ng/ml	2.5 ng/ml	3.1 ng/ml	Florant et al. (2012) and Healy and Florant (2012)
	Greater Asiatic Yellow Bat (*S. heathi*)	140 pmol/l	230 pmol/l	125 pmol/l	112 pmol/ml	125 pmol/l	Srivastava and Krishna (2007)
Insulin	American Black Bear	8.5–53 μU/ml	6.4–17 μU/ml	5.7–32 μU/ml	7.47 μU/ml	14 μU/ml	Bradford et al. (2010) and Palumbo et al. (1983)
	Marmot	12.43–37.2 μU/ml	20.1 μU/ml	13.0 μU/ml	4.31–10.3 μU/ml	4.93 μU/ml	Florant et al. (1991) and Tokuyama et al. (1991)
	Hedgehog (*E. europaeus*)	3.15 μU/ml	2.1–10.0 μU/ml			3.27–45.0 μU/ml	Laurila and Suomalainen (1974) and Hoo-Paris et al. (1978)
	Garden dormouse (*E. quercinus*)	17 μU/ml	18.5 μU/ml		20.5 μU/ml		Agid et al. (1978)
	Little Brown Bat (*M. lucifugus*)	7.29 μU/ml	16.05 μU/ml		27.43 μU/ml	5.07 μU/ml	Bauman (1990)
Glucose	American Black Bear	71–165.26 mg/dl	60–120 mg/dl		136 mg/dl	74 mg/dl	Bradford et al. (2010) and Palumbo et al. (1983)
	Marmot	154 mg/dl	135 mg/dl		131 mg/dl	137 mg/dl	Tokuyama et al. (1991)
	Golden-mantled Ground Squirrel	59 mg/dl	57 mg/dl	130 mg/dl	152 mg/dl	166–220 mg/dl	Galster and Morrison (1975)
	Little Brown Bat			28 mg/dl	155 mg/dl		Bauman (1990)
Ghrelin	Grizzly Bear (*U. arctos horribilis*)		110 pg/ml	95 pg/ml		160 pg/ml	Gardi et al. (2011)
	Marmot		4.05 ng/ml		8.20 ng/ml	9.86 ng/ml	Doherty et al.; unpublished data (2013)
	Golden-mantled Ground Squirrel	8.0–4.5 ng/ml	3.0–3.8 ng/ml		1.0–3.0 ng/ml	3.6–5.4 ng/ml	Healy et al. (2010)
Adiponectin	American Black Bear	1912 ng/ml	1333 ng/ml		1906 ng/ml		Bradford et al. (2010)
	Marmot	1.2[b]	1.0		0.7	1.2	Florant et al. (2004)
	Little Brown Bat		3–4 ng/ml				Townsend et al. (2008)
Thyroid (T_4)[c]	American Black Bear	1.2 μg/100 ml	0.8 μg/100 ml		1.6 μg/100 ml		Nelson et al. (1973)
	Woodchuck	7.5 μg	11 μg		4.5 μg		Wenberg and Holland (1973)
Glucocorticoids	American Black Bear	4.3–7.4 μg/dl	5.6–7.7 μg/dl		1.5–3.8 μg/dl	1.8–5.4 μg/dl	Palumbo et al. (1983), Donahue et al. (2003a, 2003b) and Hellgren et al. (1993)
	Woodchuck	9.725 ng/ml			7.325 ng/ml		Florant and Weitzman (1980)
	Marmot		10 ng/ml			54 ng/ml	Kastner et al. (1977) and Kastner et al. (1978)
	European Ground Squirrel (*S. citellus*)	141.2 nmol/l	49.97 nmol/l	138.3 nmol/l	170 nmol/l	225.4 nmol/l	Shivatcheva et al. (1988)
	Little Brown Bat	0.435 μg/dl	0.87 μg/dl		0.525 μg/dl	0.41 μg/dl	Gustafson and Belt (1981)

[a]This table reflects a summary of key studies in hibernation with potential insight into bone metabolism. The table is not intended to be all inclusive and the ranges given do not highlight seasonal changes that may be found in particular studies.

[b]Protein expression of serum adiponectin.

[c]T_4 concentration is reported for comparison between studies because T_3 was not investigated in both studies.

Table 2 Summary of two neuroendocrine factors influencing bone and fat metabolism in hibernators[a]

		Seasonal concentrations					
			Winter (hibernation)				
Hormone	Species	Fall (pre-hibernation)	Early hibernation	Late hibernation	Spring (post-hibernation)	Summer (active)	References
NPY	American Black Bear (*U. americanus*)	211.9 pmol/l		226.17 pmol/l	192.49 pmol/l		Bradford et al. (2010)
	Daurian Ground squirrel (*S. dauricus*)	0.70RU[b]		0.75RU	1.0RU	0.56RU	Xing et al. (2012)
	Greater Egyptian Jerboa (*J. orientalis*)			23.9RU		10.4RU	El Ouezzani et al. (2001)
	Greater Mouse-tailed Bat (*R. microphyllum*)	2.8 pg/mg[b]			1.25 pg/mg	3.5 pg/mg	Levin et al. (2012)
Serotonin	American Black Bear	753.1 ng/ml		600.95 ng/ml	689.55 ng/ml		Bradford et al. (2010)
	Woodchuck (*M. monax*)	5.0 ng/mg[c]		5.1 ng/mg	5.3 ng/mg		Young et al. (1979)
	Marmot (*M. flaviventris*)			10.4 nM		1.1 nM	Reid et al. (1992)
	Golden-mantled Ground Squirrel (*C. lateralis*)			0.30–0.40 µg/g		0.40–0.70 µg/g	Spafford and Pengelley (1971)
	Little Brown Bat (*M. lucifugus*)	4.15 µg/g[c]	11.44 µg/g	4.94 µg/g	4.05 µg/g		Haymovits et al. (1976)

[a]This table reflects a summary of key studies in hibernation with potential insight into bone metabolism. The table is not intended to be all inclusive and the ranges given do not highlight seasonal changes that may be found in particular studies.
[b]Denotes RNA gene expression.
[c]Protein expression of serum adiponectin.

Table 3 Summary of some of the skeletal factors influencing energy and fat metabolism in hibernators[a]

		Seasonal concentrations				
Hormone	Species	Fall (pre-hibernation)	Winter (hibernation)	Spring (post-hibernation)	Summer (active)	References
Osteocalcin	American Black Bear (*U. americanus*)	16.9 ng/ml	27.7–70.4 ng/ml	49.3 ng/ml	51.2 ng/ml	Donahue et al. (2006a, 2006b) and Vestergaard et al. (2011)
	Woodchuck (*M. monax*)	93.1 ng/ml	139.72 ng/ml	192.13 ng/ml		Doherty (2013)
PTH	American Black Bear	14.6 pg/ml	25.3–36.66 pg/ml	41.4 pg/ml	41.36 pg/ml	Donahue et al. (2006a, 2006b) and Vestergaard et al. (2011)
$1,25(OH)_2D$	American Black Bear and Brown Bear (*U. arctos*)		71.97–88.6 pmol/l		103.09–192.5 pmol/l	Vestergaard et al. (2011) and Seger et al. (2011)

[a]This table reflects a summary of key studies in hibernation with potential insight into bone metabolism. The table is not intended to be all inclusive and the ranges given do not highlight seasonal changes that may be found in particular studies.

Table 4 Summary of some reproductive factors influencing bone and fat metabolism in hibernators[a]

		Seasonal concentrations					
			Winter (hibernation)				
Hormone	Species	Fall (pre-hibernation)	Early hibernation	Late hibernation	Spring (post-hibernation)	Summer (active)	References
Estrogens	American Black Bear (*U. americanus*)	19–25 pg/ml		25–27 pg/ml	20–44 pg/ml	44–70 pg/ml	Tsubota et al. (1997)
	European Ground Squirrel (*S. citellus*)	120 pg/ml			198 pg/ml	330 pg/ml	Millesi et al. (2008)
	Greater Asiatic Yellow Bat (*S. heathi*)	250.43 pg/ml	197.9 pg/ml	51.9 pg/ml	182.1 pg/ml	119.05 pg/ml	Krishna and Abhilasha (2000)
Prolactin	Japanese Black Bear (*U. thibetanus japonicas*)	2.24 ng/ml	7.96 ng/ml	8.14 ng/ml	8.4–9.1 ng/ml	8.67 ng/ml	Sato et al. (2001) and Howell-Skalla et al. (2000)
	Woodchuck (*M. monax*)	0.2–1.3 ng/ml		2.35 ng/ml	7.0–20.7 ng/ml		Concannon et al. (1999)
Testosterone	American Black Bear	0.1–2.0 ng/ml	1.0 ng/ml	2.4 ng/ml	0.6–12.4 ng/ml	12.4–63.2 ng/ml	Tsubota et al. (1997) and Tsubota et al. (1999)
	Woodchuck	0.1–0.2 ng/ml	1.0 ng/ml	3.4–6.6 ng/ml	0.6–1.0 ng/ml	0.1–0.2 ng/ml	Concannon et al. (1999) and Baldwin et al. (1985)
	Hedgehog (*E. europaeus*)	1.33 pg/ml		17.9 pg/ml	17.9 pg/ml		Saboureau (1986)
	Golden-mantled Ground Squirrel (*C. lateralis*)	0.061 ng/ml		0.063 ng/ml	8.824 ng/ml		Barnes (1986)

[a]This table reflects a summary of key studies in hibernation with potential insight into bone metabolism. The table is not intended to be all inclusive and the ranges given do not highlight seasonal changes that may be found in particular studies.

levels of leptin inhibit NPY/AGRP while stimulating pro-opiomelanocortin (POMC) and cocaine-amphetamine regulated transcript (CART) to reduce food intake and increase energy expenditure (Schwartz et al. 1997; Kristensen et al. 1998). Furthermore, leptin works in association with insulin and glucocorticoids in regulating food intake and body mass (Bornstein et al. 1997; Inui 1999).

Leptin is also a regulator of bone remodeling through multiple pathways (Lee and Karsenty 2008). To influence bone remodeling, leptin acts primarily through the ventromedial nuclei to stimulate osteoblastic β2-adrenergic receptors (Adrβr), and thereby up-regulate osteoclastogenesis and bone resorption via receptor activator of nuclear factor-Kβ ligand (RANKL) (Takeda et al. 2002). Leptin also acts through the arcuate nuclei in a separate pathway to induce production of CART which inhibits bone resorption (Elefteriou et al. 2005). The role of leptin on bone, therefore, is complex and may contribute to the regulation of balanced bone remodeling during hibernation.

To prepare for hibernation, food intake is increased in the summer to build fat stores. Hibernators gradually reduce feeding and metabolic rate during the summer-to-fall transition to prepare for hibernation (Davis 1976; Ward and Armitage 1981; Hissa et al. 1998). Typically, serum leptin peaks in the fall and hibernators are believed to experience hyperleptinemia before they stop eating to allow sufficient accumulation of fat for the winter (Kronfeld-Schor et al. 2000; Florant et al. 2004). Well into the hibernation season (November and December), the level of leptin begins to drop, although in marmots it remains elevated above spring concentrations until late February (Florant et al. 2004). High leptin concentrations during pre-hibernation and early hibernation is found across various species, including bears, woodchucks, marmots, and ground squirrels (*Callospermophilus lateralis*) (Concannon et al. 2001; Florant et al. 2004, 2012; Donahue et al. 2006b; Seger et al. 2011; Healy and Florant 2012; Doherty 2013). Little brown bats (*Myotis lucifugus*) decrease leptin and ObR expression transiently during periods of greatest gain of fat in the summer, but these subsequently rise in the pre-hibernation season (Townsend et al. 2006). This pattern has also been reported in greater Asiatic yellow bats (*Scotophilus heathi*) with the highest leptin concentrations reached in late fall to early winter (Srivastava and Krishna 2007). Leptin protein isoforms appear to be structurally different between hibernating and active bats, indicating that leptin may have an altered role during hibernation (He et al. 2010).

Considering that obesity leads to high circulating leptin, hibernators would seem to be at risk for fragile bones and fractures with repeated seasonal obesity. However, impairment of leptin signaling during hyperleptinemia in the fall may provide some protection against bone loss in hibernators. Indeed, it has been suggested that resistance to leptin and the subsequent inability of leptin to elicit bone remodeling is a protective factor for bone in obese individuals (Ducy et al. 2000; Schilling et al. 2001; Takeda et al. 2002). A potential mechanism through which leptin could exert a positive influence on bone during hyperleptinemia and impaired transport across the BBB is through arcuate projections into circulation which express ObR (Cheunsuang and Morris 2005). Activation of CART through this route could reduce bone resorption by decreasing production of osteoblastic RANKL (Elefteriou et al. 2005).

Insulin and glucose

Mammalian hibernators have high insulin levels in their serum in the fall relative to summer (Florant and Bauman 1984; Florant et al. 1985, 1991; Mrosovsky and Boshes 1986). These animals experience seasonal hyperinsulinemia, hyperglycemia, and peripheral resistance to insulin. High circulating insulin concentration is related to the reduced ability of fat and muscle cells to take up glucose, causing further stimulation of pancreatic β-cells to release insulin (Florant and Bauman 1984; Hoo-Paris et al. 1984), similar to insulin resistance observed in humans (Flier et al. 1982). Insulin's reduced ability to promote glucose uptake in hibernators in the fall may be related to changes in muscle and fat-cell processes that involve glucose transporter function (Hoehn et al. 2004). The reasons why hibernators do not become overtly diabetic most likely reside in their ability to stop eating for prolonged periods.

In greater Asiatic yellow bats, melatonin has been shown to enhance glucose clearance in the blood during pre-hibernation when the animals are at their maximum fat mass, and it may also protect the animal from hypoglycemia during hibernation (Srivastava and Krishna 2010). Furthermore, increased melatonin concentrations during hibernation compared to summer levels in brown bears (*U. arctos*) suggest that this hormone may play a role in metabolic suppression during the winter (Ware et al. 2013). What turns off a hibernator's appetite and thus reduces circulating glucose to basal levels is unknown, but recent studies suggest that AMPK, NPY, and NPY-Y1 receptors, in addition to melatonin, may be involved in the mechanism (Dark 2005; Dark and Pelz 2008; Swoap 2008; Florant et al. 2010). Interestingly, melatonin decreases RANKL-mediated osteoclastogenesis to result in increased bone mass (Koyama et al. 2002); however, the role of melatonin on bone in hibernating animals has not been investigated.

During hibernation, insulin concentrations are low in hedgehogs (*Erinaceus europaeus*) and 13-lined ground squirrels (*Spermophilus tridecemlineatus*; Laurila and Suomalainen 1974; Agid et al. 1978; Hoo-Paris et al. 1978). Marmots' insulin levels are highest in the plasma in September through October and begin to decline throughout hibernation to reach low concentrations in the spring (Florant et al. 1991; Tokuyama et al. 1991). However, insulin levels remain unchanged from active periods to torpor in garden dormice (*Eliomys quercinus*) and edible dormice (*Glis glis*; Agid et al. 1978; Castex et al. 1984). In the American black bear, insulin decreased during hibernation (Bradford et al. 2010) or did not change compared to active seasons (Palumbo et al. 1983). Only bats (*M. lucifugus* and *S. heathi*) experienced increasing insulin during the early phase of winter dormancy (Bauman 1990; Srivastava and Krishna 2007).

In skeletal signaling, insulin binds its receptor on osteoblasts, decreasing osteoprotegerin (OPG) expression, which allows increased binding of RANKL and promotion of osteoclastic bone resorption (Elefteriou et al. 2005; Ferron et al. 2010). The acidic environment of resorption lacunae (pH 4.5) effectively decarboxylates osteocalcin (ucOC) to release it from the bone matrix. This biologically active ucOC increases adiponectin, proliferation of β-cells, production of insulin, and a sensitivity to insulin that causes a positive feedback loop between insulin and ucOC (Ferron et al. 2010). In mice, ucOC stimulates the secretion and sensitivity of insulin and increases energy expenditure by muscles (Lee and Karsenty 2008).

Paradoxically, hibernating American black bears have increased total serum osteocalcin, but decreased levels of serum insulin (Bradford et al. 2010). Woodchucks also experience high total osteocalcin levels throughout the hibernation season and spring (Doherty 2013). However, a separate study found the opposite in brown bears and reported decreased osteocalcin concentrations during hibernation (Vestergaard et al. 2011). Varying osteocalcin concentrations in different studies may be a result of unidentified aspects of hibernation physiology, species-specific differences in osteocalcin carboxylation, and/or difficulties in identifying osteocalcin fragments (Booth et al. 2013). Low levels of serum

insulin in bears are likely the result of their fasting state during hibernation. Since ucOC regulates insulin sensitivity independently of its effect on the secretion of insulin (Lee et al. 2007), bears may compensate for lower insulin levels by increasing their sensitivity to insulin via increased osteocalcin. Because osteocalcin is eliminated through renal degradation and urinary removal (Delmas et al. 1983), high levels of serum osteocalcin is likely caused by reduced renal function while bears are anuric during hibernation.

Given varying concentration patterns in different species during hibernation, insulin may play different roles in regulating bone in hibernators. Reduction of insulin during hibernation may provide a protective function against bone resorption in bears, hedgehogs, marmots, and ground squirrels. However, unchanging levels in other hibernating species, especially during periods of reduced serum glucose, suggest bone resorption would be favored over bone formation. Therefore, insulin may have alternate roles in bone metabolism in different hibernating species.

Ghrelin

Ghrelin is an orexigenic hormone working in opposition to leptin (Hewson et al. 2002; Kim et al. 2004). Fasting increases ghrelin expression from the stomach (Toshinai et al. 2001), although it is produced in a number of other tissues (Kojima et al. 1999). Peripheral and intercerebroventricular administration of exogenous ghrelin increase food intake and weight gain (Tschop et al. 2000; Keen-Rhinehart and Bartness 2005), but ghrelin action can be overridden by central injection of leptin or insulin (Hewson et al. 2002). Ghrelin acts through the hypothalamus to stimulate production of NPY and AGRP while inhibiting POMC to balance energy homeostasis (Cowley et al. 2003; Chen et al. 2004). Considering its production by multiple tissues and its close association with leptin and insulin, it is not surprising that ghrelin has a role in coupling bone and energy metabolism (Delhanty et al. 2014). In fact, osteoblasts themselves produce ghrelin (Delhanty et al. 2006). In the skeleton, ghrelin promotes osteoblast proliferation by stimulating the phosphoinositide-3 kinase and mitogen-activated protein kinase pathways through an unknown mechanism (Delhanty et al. 2006). Central and peripheral administration of ghrelin increase bone mineral density in rats (Fukushima et al. 2005; Choi et al. 2013), and osteoblast apoptosis is suppressed by ghrelin *in vitro* (Kim et al. 2005), indicating that ghrelin positively regulates bone formation.

In hibernating grizzly bears (*U. arctos horribilis*) ghrelin levels in the serum are significantly lower than during summer (Gardi et al. 2011). Low circulating ghrelin is also documented in hibernating marmots and golden-mantled ground squirrels (Healy et al. 2010; Doherty et al. unpublished data 2013). The highest levels of plasma ghrelin in golden-mantled ground squirrels occurs during the hyperphagic period in the fall, indicative of the gain in fat observed in the fall in these animals (Healy et al. 2010). Furthermore, low concentrations of ghelin in the plasma during the winter are hypothesized to contribute to suppression of appetite throughout hibernation. Peripheral administration of ghrelin to golden-mantled ground squirrels during hibernation increases food intake and physical activity (Healy et al. 2011). Considering that the level of circulating ghrelin is significantly lower during hibernation in bears, marmots, and ground squirrels, it is unlikely that ghrelin has a prominent role in maintaining bone through the winter when these animals are physically inactive. However, the direct effects of ghrelin on bone in a hibernating animal remain to be investigated.

Adiponectin

Like leptin, adiponectin is a fat-derived hormone that increases expenditure of energy and heightens sensitivity to insulin, thereby reducing body weight (Berg et al. 2002; Qi et al. 2004). Obese and insulin-resistant animals experience decreased adiponectin levels (Havel 2002), indicating that adiponectin works in concert with leptin and insulin to regulate energy metabolism. Expression of adiponectin and its receptors (AdipoR1 and AdipoR2) have been identified in osteoblasts and osteoclasts, suggesting paracrine and/or autocrine action (Berner et al. 2004; Shinoda et al. 2006). Adiponectin-transfected mice fed a normal diet experienced an increase in trabecular bone mass and suppressed osteoclastogenesis while plasma glucose and insulin concentrations did not change (Oshima et al. 2005). Thus, adiponectin may affect bone independent of insulin and energy homeostasis. Moreover, adiponectin is structurally similar to RANKL, OPG, and TNFα and inhibits activation of the cAMP-PKA pathway by NF-ĸB (Ouchi et al. 2000; Berner et al. 2004), further supporting its negative effects on osteoclastogenesis. Osteocalcin also stimulates production of adiponectin by adipocytes, thereby promoting insulin sensitivity in a positive feed-back loop further linking bone and energy metabolism (Lee et al. 2007).

Animals seeking to gain significant body mass for hibernation (e.g., marmots and bats) demonstrate

high adiponectin levels in the summer and decreased levels in the fall when leptin and insulin levels are highest (Florant et al. 2004; Townsend et al. 2008). This pattern occurs in concert with the accumulation of lipid in the fall until the intake of food ceases entirely and lipolysis begins. American black bears experience low adiponectin concentrations during hibernation (Bradford 2010). Low adiponectin levels are consistent with the requirement for conserving energy during hibernation; therefore, any protective function adiponectin may have on bone is likely second to the metabolic actions of this hormone.

Thyroid hormone

Thyroid hormone has multiple effects in the body across several systems beyond the scope of this review. However, one of the most prominent effects of thyroid hormone is on metabolism in which oxygen consumption is increased, thereby stimulating the turnover of glucose and lipid, as well as the promotion of heat production (Berne et al. 2004). Thyroid hormone is important in the growth and maturation of bone and influences bone remodeling in non-hibernating adults. Triiodothyronine (T_3), the most biologically active of the thyroid compounds, regulates both bone formation and bone resorption through the downstream release of IGF-1 and interleukin 6 and 8, respectively (Berne et al. 2004).

The metabolic and T_b functions of thyroid hormone make it a critical factor in hibernation studies. Its relation to bone remodeling bolsters the link between fat and skeletal metabolism. In the American black bear, T_3 and thyroxine (T_4) serum concentrations are significantly lower during hibernation (Nelson et al. 1973; Azizi et al. 1979; Tomasi et al. 1998). This pattern is different, however, in woodchucks which experience increased serum T_4 levels during hibernation compared to pre-hibernation and post-hibernation seasons (Wenberg and Holland 1973). In support of these findings, plasma T_4 and T_3 concentrations are lowest during the early summer months in woodchucks and begin to increase in the pre-hibernation season (Young 1984). Thus, the action of thyroid hormone on the skeleton of hibernators may function differently among species and requires further investigation.

Cortisol

Endogenous glucocorticoids serve a number of important functions including increasing protein catabolism and gluconeogenesis in the liver, producing and reserving carbohydrates during periods of fasting, and promoting conversion of glucose and mobilization of fat (McCarthy et al. 1990; Canalis 2005; Peckett et al. 2011). Direct effects of glucocorticoids on bone include stimulating RANKL while inhibiting OPG production by osteoblasts (Hofbauer et al. 1999), effectively increasing osteoclastogenesis and osteoclast activity. In contrast, glucocorticoids also mediate apoptosis of osteoclasts (Tobias and Chambers 1989; Dempster et al. 1997) and reduce their number (Lindgren et al. 1983); however, long-term glucocorticoid treatment associated with bone loss is well documented (Kanis et al. 2007).

Cortisol is the primary glucocorticoid produced in the adrenal cortex in many species, including marmots and bears (Kastner et al. 1977; Donahue et al. 2003b). In bears, cortisol increases significantly during hibernation (Palumbo et al. 1983; Harlow et al. 1990; Hellgren et al. 1993; Donahue et al. 2003a, 2003b). Wild American black bears have 28–39% higher average cortisol levels in the winter and spring compared to the summer and fall (Harlow et al. 1990). Hibernating little brown bats also have high circulating cortisol concentrations compared to active bats (Gustafson and Belt 1981). Unlike bears and bats, however, plasma cortisol decreased in marmots and European ground squirrels (*Spermophilus citellus*) during hibernation (Kastner et al. 1978; Shivatcheva et al. 1988; Tokuyama et al. 1991), but woodchucks did not experience changing cortisol levels between seasons despite a significant circadian rhythm (Florant and Weitzman 1980).

High levels of glucocorticoids during hibernation may contribute to a reduced number of osteoclasts by promoting apoptosis. This promotes reduced and balanced bone remodeling by decreasing resorption; however, glucocorticoid stimulation of RANKL in non-hibernating animals suggests that bone loss would be the primary outcome of increased cortisol during hibernation. Different seasonal patterns of cortisol concentrations among hibernators indicate that this hormone and its derivatives may have species-specific bone-related functions.

Neuropeptide-Y

NPY positively regulates the intake of food and the storage of fat. In bone marrow, NPY is produced by megakaryocytes, and NPY-immunoreactive fibers have been identified in bone lining and marrow cells. Similarly, NPY is expressed in osteoblasts, osteocytes, and potentially in osteoclasts (Igwe et al. 2009; Khor and Baldock 2012) in addition to a number of peripheral organs (e.g., pancreas and adrenal medulla). Furthermore, the distribution of NPY receptors spans multiple tissues, indicating a pronounced circulating role for NPY (Khor and Baldock 2012). NPY acts downstream of leptin and its production is

inhibited by central leptin signaling which contributes to the homeostatic response to stimulate appetite and conserve energy (Allison et al. 2007). However, NPY acts independently of leptin in regulating bone metabolism (Ducy et al. 2000).

Like many neuroendocrine factors, the importance of NPY on the regulation of bone is complex but becoming increasingly apparent. When NPY is centrally over-expressed in mice, the volume and mass of vertebral bone decrease significantly (Ducy et al. 2000). In knockout mice, both NPY−\− and Y2−\− receptor in the hypothalamus, cause an increase in the formation and mineralization of bone (Allison and Herzog 2006; Allison et al. 2007; Baldock et al. 2009). mRNA levels for the Y2 receptor were not detected in bone suggesting a centrally mediated effect (Allison and Herzog 2006), however, NPY Y1 receptors on osteoblasts suggest that peripheral NPY may mediate bone metabolism (Baldock et al. 2007; Lundberg et al. 2007).

In grizzly bears, plasma NPY concentrations remained the same between hibernation and periods of activity (Gardi et al. 2011). Similarly, NPY mRNA expression patterns in the brains of hibernating and active ground squirrels (*S. tridecemlineatus*, *Spermophilus richardsonii*, and *Spermophilus dauricus*) are not different (Reuss et al. 1990; Xing et al. 2012). In greater mouse-tailed bats (*Rhinopoma microphyllum*) increased hypothalamic NPY mRNA expression corresponded with a high demand for increased mass of fat during summer (Levin et al. 2012). Interestingly, increased NPY mRNA expression was hypothesized to stimulate food intake during seasonal resistance to leptin associated with pre-hibernation. In a separate study in little brown bats, NPY expression remained elevated throughout hibernation (Laemle and Cotter 1992). Increased NPY expression (mRNA) was also found in hibernating jerboas (*Jaculus orientalis*) (El Ouezzani et al. 2001) and the serum of American black bears (Bradford 2010) in comparison to animals in summer. Of additional significance, NPY receptor Y1 may induce reduction of T_b associated with torpor (Dark and Pelz 2008). Thus, NPY may have different bone-related functions in different hibernating species and requires further investigation.

Serotonin

Peripheral serotonin is primarily produced in the gut by enterochromaffin cells and is involved in numerous aspects of digestion, food intake, and a host of other metabolic functions (Berger et al. 2009). Central serotonin acts to decrease appetite and inhibit gain in weight (Blundell 1984). Functional serotonin receptors exist in osteoblasts and osteocytes (Westbroek et al. 2001), and gut-derived serotonin may play a negative role in the maintenance of bone (Yadav et al. 2009). On the other hand, brain-derived serotonin cannot cross the BBB, and it acts on ventromedial hypothalamic neurons to promote bone formation in the absence of leptin (Yadav et al. 2009).

It is unknown if hyperleptinemia coupled with endogenous levels of serotonin during hibernation would be able to elicit changes in bone despite hibernation. Serum serotonin in American black bears is highest during pre-hibernation, decreases in hibernation, and begins to increase again in the post-hibernation season (Bradford et al. 2010). In woodchucks, however, serotonin concentrations in cerebral spinal fluid do not change significantly between seasons (Young et al. 1979), yet serotonin in cerebral spinal fluid in marmots is reported to be significantly higher during hibernation (Reid et al. 1992). Serotonin levels in the brain are lowest during entrance into hibernation in golden-mantled ground squirrels (Spafford and Pengelley 1971) compared to decreased serotonin protein expression in late hibernation experienced by little brown bats (Haymovits et al. 1976). Low levels of gut-derived serotonin during hibernation may result from decreased intestinal activity and may reduce the negative influence of peripheral serotonin on bone to contribute to the protection of bone during hibernation.

Parathyroid hormone

A calcitropic hormone, PTH works in conjunction with calcitonin to maintain serum calcium levels within a narrow physiological range. PTH promotes mobilization of calcium from bone by increasing bone resorption and the kidney's production of 1,25-dihydroxyvitamin D in conditions of serum hypocalcemia (de Paula and Rosen 2010). Both single-dose and intermittent PTH treatments stimulate lipolysis of white adipose tissue and the production of glycerol in a dose-dependent manner, also linking PTH and adiposity (Gozariu et al. 1974; Sinha et al. 1976). Intermittent PTH treatment inhibits the differentiation of adipocytes (Rickard et al. 2006; Kulkarni et al. 2007). Evidence suggests that high PTH levels may contribute to decreased sensitivity to insulin (Kumar et al. 1994; Chiu et al. 2000), although other studies found no relationship between these hormones (Anastasilakis et al. 2008). Regardless, individuals with high body mass have high circulating PTH (Snijder et al. 2005; Ishimura et al. 2013).

In American black bears, PTH levels increased during hibernation and peaked in the post-hibernation season relative to pre-hibernation (Donahue et al. 2006a). Increased PTH during hibernation and post-hibernation would be expected to signal the release of calcium from the bone matrix, but bone resorption decreases (McGee et al. 2008; Bradford 2010) and serum calcium is unchanged (Floyd et al. 1990; Bradford 2010). However, the PTH assay used in these studies detects large C-terminal PTH (C-PTH) fragments in addition to the full protein (PTH 1–84). Large C-PTH fragments (e.g., 7–84) may have anti-resorptive effects on bone (Divieti et al. 2002; Langub et al. 2003). Considering that C-PTH fragments are produced in greater proportion than intact PTH (1–84) during renal failure in humans (Brossard et al. 1996), they may provide a protective function to bone by reducing bone resorption during hibernation (McGee et al. 2008).

Patients with primary hyperparathyroidism experience significant reductions in the cortical thickness of the iliac crest, while trabecular number increases compared to controls (Bilezikian et al. 1991). However, mild hyperparathyroidism may have a protective effect against post-menopausal osteoporosis (Dempster et al. 1999). Thus, PTH may act as an anabolic agent that helps preserve bone during hibernation in a similar manner as in post-menopausal women with mild hyperparathyroidism. PTH also promotes renal reabsorption of calcium; this may contribute to eucalcemia. Other cross-sectional and longitudinal studies in bears, however, indicate that there is no difference between seasonal PTH concentrations (Seger 2008; Seger et al. 2011; Vestergaard et al. 2011).

Vitamin D

Vitamin D_3 is obtained through diet or from exposure of skin or fur to ultraviolet light (Vieth 1999; Hymoller and Jensen 2010). Vitamin D_3 is converted into the active form of vitamin D, calcitriol, or 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), by hydroxylation of 25(OH)D in the kidney. Calcitriol is responsible for homeostasis of calcium and phosphate, maintaining normal bone turnover, and mineralization of the organic matrix by controlling gastrointestinal absorption of calcium, excretion of renal calcium and bone turnover. Vitamin D is also inversely associated with adiposity (Snijder et al. 2005).

Lower levels of $1,25(OH)_2D$ are expected during hibernation because of reduced exposure to sun. The serum concentration of 25(OH)D was reported to be higher or unchanged in hibernating American black bears compared to non-hibernating bears (Donahue et al. 2006a; Seger 2008). However, bears' concentrations of serum calcitriol were higher post-hibernation compared to hibernation (Seger et al. 2011; Vestergaard et al. 2011). Increased calcitriol is likely a consequence of the need for increased intestinal absorption of calcium when feeding resumes following hibernation. The low levels of calcitriol during hibernation may contribute to lower rates of bone remodeling (i.e., bone formation and resorption).

Role of sex hormones in bone and fat metabolism

Bone and adipose tissues also are greatly influenced by hormones of reproduction. Estrogen and androgen receptors (ER and AR, respectively) are found throughout bone and fat tissue and mediate the effects of sex hormones in conjunction with other endocrine factors (e.g., PTH), resulting in highly complex regulation of interrelated systems. Of considerable importance is the task of determining different mechanisms of these systems in mammalian hibernators compared to non-hibernating species as an evolutionary means of successfully surviving prolonged shortages in resources and extreme temperatures, and then be able to reproduce after hibernation. The literature covering sex hormones in hibernators is quite extensive and this review touches only on key points that relate to skeletal regulation.

Estrogens

Estradiol 17β is an estrogen produced by the sex organs, adrenal glands, or converted from testosterone by the P450 aromatase enzyme (Venken et al. 2008). Estrogen deficiency (e.g., ovariectomy) increases the mass of fat and the concentration of hypothalamic NPY, while decreasing sensitivity to leptin (Ainslie et al. 2001). Treatment with exogenous estrogen returned NPY levels to normal, reduced bone loss, and reduced the accumulation of fat (Malluche et al. 1986; Hill et al. 2008; Zengin et al. 2010). Effects of estrogen deficiency in bone include increased bone remodeling and decreased bone mineral density (Tozum et al. 2004). Additional osteoclast progenitors may result from increased production of osteoclastogenic cytokines that are normally suppressed by estrogens (Di Gregorio et al. 2001). Estradiol 17β can suppress osteoclastogenesis by enhancing the production of OPG and by decreasing receptor activator of RANKL in osteoblasts (Venken et al. 2008).

Estradiol 17β in male American black bears decreased from December to March (Tsubota et al. 1999), which may be the result of decreased testosterone production during denning and less testosterone being converted to estradiol. Estradiol 17β also

has been measured in 23 captive adult female Japanese black bears (*Ursus thibetanus japonicus*), eight of which gave birth (Sato et al. 2000). The lowest levels of estradiol were observed in December with rises in January when most bears gave birth. Thus, low concentrations of estrogen would seemingly cause these animals to experience increased resistance to leptin, NPY levels, and bone resorption during hibernation. These patterns are not seen in association with the low estrogen levels reported during hibernation, but rather in the pre-hibernation season when hibernators are gaining fat in preparation for winter. European ground squirrels experience the highest levels of estradiol post-lactation, indicative of two annual estrus cycles (Millesi et al. 2008). Greater Asiatic yellow bats have low concentrations of estradiol during the ovulatory delay experienced during winter dormancy, December through mid-February (Krishna and Abhilasha 2000). This, however, was preceded and followed by high estradiol levels associated with recrudescence (October–November) and renewal of ovarian activity (mid-February through early March). Therefore, the effects of estrogen may be seasonally uncoupled from its influence on the metabolism both of bone and fat in hibernating mammals, as indicated by species-specific reproductive cycles.

Prolactin

Prolactin is a hormone that is produced by the lactotrope cells of the anterior pituitary gland (Seriwatanachai et al. 2009). It is responsible for the production of breast milk in postpartum women, although it also has an active role in the inhibition of adipocyte production of leptin and lipolysis in male and female rats (Brandebourg et al. 2007; Seriwatanachai et al. 2009). During pregnancy, prolactin causes a high turnover of bone that provides calcium for fetal growth and for production of breast milk (Seriwatanachai et al. 2008). Continued exposure to prolactin (e.g., during lactation), increases bone turnover, loss of calcium, osteopenia, and osteoporosis (Seriwatanachai et al. 2008).

The projected effects of prolactin on the health of bones are potentially significant in seasonally inactive, hibernating, female animals. The concentration of prolactin increases in December, 1 month prior to parturition (January to early February), relative to pre-hibernation in pregnant Japanese black bears and continues to rise post-parturition (Sato et al. 2001). Interestingly, prolactin is also high in the spring compared to other seasons in non-pregnant female bears. American male black bears experience little change in prolactin levels during hibernation (Tsubota et al. 1999); however, they increase significantly March through June (Howell-Skalla et al. 2000). Changes in prolactin concentrations in male marmots are linked to seasonal changes in photoperiod (Concannon et al. 1999). Increased prolactin may contribute to increased bone resorption in pregnant and lactating females bears during hibernation and to increased resorption in all bears, including males, following hibernation.

Testosterone

Testosterone is a sex hormone produced in the testicles of males and in the ovaries of females. In addition to its role as a sex hormone, testosterone has profound effects in fat and bone. Low testosterone contributes to the metabolic syndrome by influencing metabolic hormones, such as insulin, leptin, adiponectin, and ghrelin, to favor increasing adiposity (De Maddalena et al. 2012). To impact bone remodeling, testosterone is aromatized to estrogen and acts to increase the lifespan of osteoblasts and osteoclasts by preventing apoptosis (Riggs et al. 2002). In addition, there are receptors for testosterone on osteoblasts and osteoclasts (Vanderschueren and Vandenput 2000; Kung 2003). *In vitro* testosterone increases the proliferation and differentiation of osteoblast-like cells (Kung 2003), and stimulation of ARs in osteoblastic stromal cells suppresses osteoclast differentiation in bone marrow (Vanderschueren and Vandenput 2000). Testosterone also increases periosteal bone formation to enhance the overall mechanical strength of bone (Vanderschueren and Vandenput 2000; Riggs et al. 2002; Kung 2003). Interestingly, biosynthesis of testosterone is promoted by the osteocalcin receptor, GPRC6A, found in testes (Oury et al. 2011).

Hibernating male rodents experience involution of the testes throughout the dormant period. As hibernators emerge from hibernation, testicular activity resumes in preparation for mating (McMillin et al. 1976; Baldwin et al. 1985; Barnes 1986; Saboureau 1986; Barnes et al. 1988; Concannon et al. 1999). In American black bears, testicular recrudescence is experienced January through May, reflecting the later breeding season (May through July) in these animals (Howell-Skalla et al. 2000). Serum testosterone in sexually mature, male, captive and free-ranging American black bears remains rather constant year-round except for peaks in the spring (Tsubota et al. 1997, 1999; Howell-Skalla et al. 2000). The increase in testosterone in male animals in the spring is expected to have positive impacts on bone; however, there does not appear to be a large difference

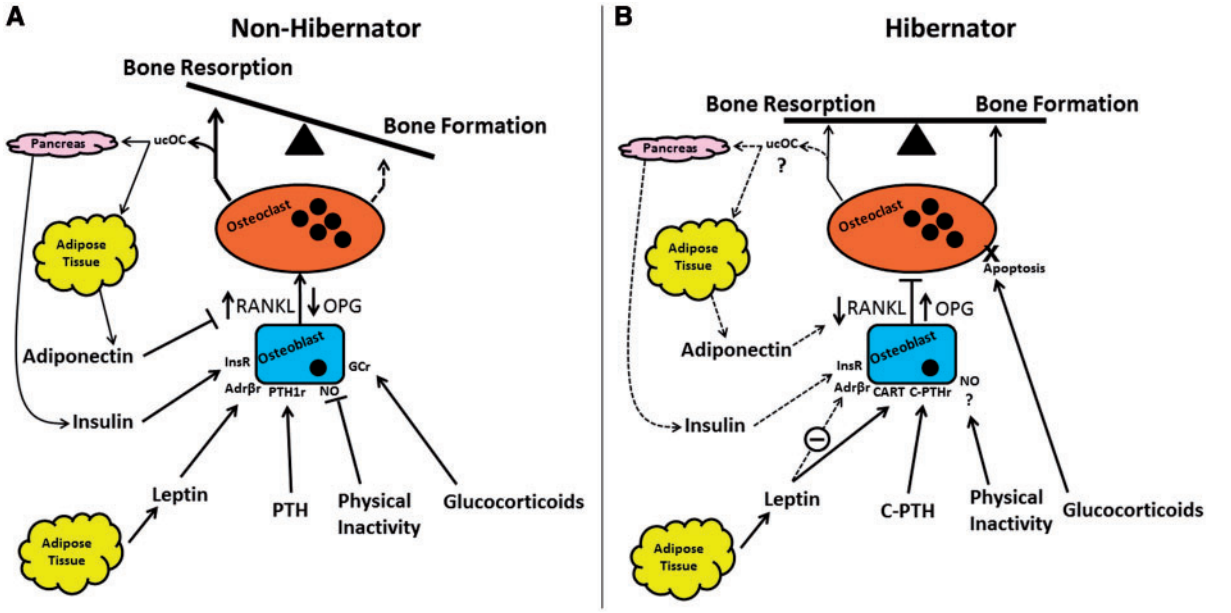

Fig. 2 Some of the factors that influence osteoclastogenesis, osteoclast activity, and bone resorption in non-hibernators **(A)** compared to hibernators **(B)**. InsR, insulin receptor; C-PTHr, C-PTH fragment receptor; GCr, glucocorticoid receptor; ucOC, uncarboxylated osteocalcin. Dashed lines represent reduced effects on bone in each pathway due to lowered bioactivity (e.g., adiponectin), impaired function (e.g., hyperinsulinemia, hyperleptinemia), or unbalanced processes (e.g., bone formation in non-hibernators during periods of high bone resorption). Arrows: activation; blunted arrows: blocked action; circled dash: impaired function; ?: mechanism still unknown; X: cell death.

between the sexes in the properties of bone in most hibernating animals. Therefore, the biological role of testosterone in bone metabolism of hibernating animals remains to be defined for both sexes.

Summary and conclusions

It is apparent that, at least for bears, marmots, woodchucks, and ground squirrels, a general pattern of bone maintenance and eucalcemia can be explained by the lack of an increase in bone resorption that would otherwise be expected in an obese and physically inactive non-hibernating animal. The lack of a mechanical loading signal in non-hibernating animals results in the inhibition of nitric oxide (NO) which stimulates increased production of osteoblastic RANKL that in turn increases osteoclastogenesis, osteoclast activity, and bone resorption (Fig. 2A) (Rubin et al. 2000, 2003). The skeleton may fail to perceive the lack of loading associated with hibernation (Seger et al. 2011). This may occur through continued production of NO and subsequent inhibition of RANKL production (Fig. 2B). Furthermore, anabolic PTH mechanisms and C-terminal PTH anti-resorptive effects may contribute to preservation of bone during hibernation (Fig. 2B).

The effects of seasonal adiposity on the skeleton of hibernators have considerable implications for the plasticity of the regulatory signaling pathways linking fat and bone. Leptin, insulin, and glucocorticoids, all acting through their individual receptors, primarily increase bone resorption through the RANKL/OPG pathway (Fig. 2A). Each agent, however, may have a unique role in regulating bone in hibernators. For instance, leptin, which is elevated for some time during the hibernation season in many hibernators, may fail to transport across the BBB and exert its catabolic effects on bone. Instead, it may act through hypothalamic arcuate projections to stimulate production of CART and inhibition of RANKL (Fig. 2B). Resistance may also occur with insulin and block insulin-induced bone resorption and uncarboxylation of osteocalcin from the bone matrix in animals that do not lower insulin concentration during hibernation. High circulating levels of glucocorticoids for extended periods of time may also promote osteoclastic apoptosis, resulting in the reduction of available cells to degrade the surface of the bone and slow the overall rate of bone loss (Fig. 2B); although additional investigation of the effects of glucocorticoids on the skeleton during hibernation is required.

The role of osteocalcin in energy balance provides a strong link in the cross-talk between the skeleton and adipose tissue in mice. This positive feed-back

loop between insulin, uncarboxylated osteocalcin, and adiponectin demonstrates a mechanism regulating bone resorption, serum calcium, sensitivity to insulin, and energy balance as a whole (Fig. 2A). However, this area is still largely under-investigated and further research is required to understand the evolved mechanisms available to animals required to survive periods of extreme environmental conditions while maintaining the skeleton (Fig. 2B).

Although we propose that the complex pathways of various hormones are important in regulating bone remodeling, their actual biological contribution to the skeleton remains to be investigated in hibernators. Many of the hormones discussed above (e.g., adiponectin) have competing functions when it comes to energy demand versus skeletal remodeling. Regulation of energy requirements may take precedence over maintenance of bone during extreme environmental challenges, such as food shortage and low temperatures. In this case, endocrine and neuroendocrine functions likely override signals of mechanotransduction and skeletal metabolism is greatly reduced. Thus, care must be taken when interpreting the actions of single molecules on bone or interactions among several systems when survival is dependent on energy reserves.

The relationship between adipose tissue and bone, and the interacting neuropeptides and sex hormones involved in skeletal and fat metabolism pathways highlight the adaptation of highly coordinated endocrine functions to environmental challenges in hibernating animals. Indeed, the interconnected signaling between the metabolic systems of bone and fat is still largely being defined in non-hibernators. By understanding skeletal plasticity in mammals exposed to annual extremes in temperature and food shortages, it is possible to identify not only aspects of the evolution of hibernation itself but also the disease processes prevalent in biomedical research today.

Acknowledgments

We thank Sherri Wiseman for data collection and assistance with the manuscript.

Funding

This work was supported by Grant Number AR050420 from The National Institute of Arthritis and Musculoskeletal and Skin Diseases to S.W.D. Funding was also provided to G.L.F. by the Lipid Foundation at CSU (#645848).

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