UC Merced

UC Merced Previously Published Works

Title

Thyroid Hormone Regulation and Insulin Resistance: Insights From Animals Naturally Adapted to Fasting.

Permalink

<https://escholarship.org/uc/item/1p54b9mx>

Journal

Physiology (Bethesda, Md.), 32(2)

ISSN

1548-9213

Authors

Martinez, Bridget
Ortiz, Rudy M

Publication Date

2017-03-01

DOI

10.1152/physiol.00018.2016

Peer reviewed

Thyroid Hormone Regulation and Insulin Resistance: Insights From Animals Naturally Adapted to Fasting

Bridget Martinez and Rudy M. Ortiz

Department of Molecular & Cellular Biology,
University of California, Merced, California
bmartinez26@ucmerced.edu

The contribution of thyroidal status in insulin signaling and glucose homeostasis has been implicated as a potential pathophysiological factor in humans, but the specific mechanisms remain largely elusive. Fasting induces changes in both thyroid hormone secretion and insulin signaling. Here, we explore how mammals that undergo natural, prolonged bouts of fasting provide unique insight into evolved physiological adaptations that allow them to tolerate such conditions despite intermittent states of reversible insulin resistance. Such insights from nature may provide clues to better understand the basis of thyroidal involvement in insulin dysregulation in humans.

Food Deprivation as an Evolutionary Tactic

The life histories of several groups of mammals include periods of either short or prolonged bouts of complete food deprivation (30, 93, 94, 101, 121, 122, 137). These fasting periods are often coupled with specific life-history events, including hibernation, mating, molting, and migration (5, 30, 40, 65, 93, 94). For example, molting emperor and king penguins must remain ashore following the loss of plumage, resulting in reduced thermal capacity and inability to feed during this time (47). Adaptive fasting is therefore an inherent survival tactic and adaptation rather than a pathophysiological detriment, and is distinct from starvation. Adaptive fasting strategies are highly variable and dependent on the quantity of endogenous fuel stores. Although the lengths of fasts are variable, the physiological consequences of adaptive fasting are generally classified into specific phases that are driven largely by metabolic responses to loss of ingested nutrients. Here, we attempt to characterize these phases in animals naturally adapted to protracted periods of food deprivation in contrast to the well established and recognized phases of starvation defined by Cahill and colleagues (22). Given that fasting is also coupled with dynamic changes in endocrine signaling, especially thyroid hormone-mediated cellular events that should tightly regulate energy homeostasis (6), prolonged periods of food deprivation can also lead to intermittent insulin resistance (48), which, as a coping mechanism, enables metabolic shifts that spare glucose and lean tissue (129, 131–133). Most recently, the relationships between changes in thyroidal status and its contribution to, or regulation by, insulin

signaling and glucose homeostasis have been well established as detrimental, leading to pathophysiological conditions in humans (59, 123, 134), although the specific mechanisms remain largely elusive. Most studies to date have relied heavily on using genetically altered and physiologically modified animal models to study the effects of abnormal thyroidal status on insulin signaling in an effort to better assess the significance and contributions of these endocrine systems to diseases such as insulin resistance and Type 2 diabetes. Unfortunately, many of these imposed alterations do not often mimic normal signaling pathways that can lead to unintended perturbations in other systems, which remain uncharacterized and can confound the interpretation of the results. In our review, we examine mammals that undergo natural bouts of fasting, providing a unique insight into how animals that have evolved natural physiological adaptations tolerate and thrive during intermittent states of disrupted insulin signaling (Table 1).

Phases of Starvation vs. Adaptive Fasting

Phase I of fasting is comprised of a short period of digestive acclimatization, during which energy output and metabolic rate decrease, albeit the degree of these reductions varies with species, given that, in some mammals, metabolic rate drastically increases (11, 16, 22, 71, 72, 76). In adult male northern elephant seals, for example, breeding fasts are associated with energy expenditures that are 3.1 times the metabolic rate predicted by Kleiber (29). During *phase I*, tissues are primarily metabolizing glucose as their main fuel source, and the origin of blood glucose is considered

Table 1. A summary of human, rat, and comparative animal studies assessed

	Humans (Short-Term)	Humans (Long-Term)	Rats	Raccoon Dogs	American Bears	Black Gray Wolves	Blue Foxes	Sub-Antarctic Fur Seal	King Penguins	Northern Elephant Seals
Fasting time	4–10 days	33 days	2 days	4 and 8 wk	4 mo	10 days	22 days	15–73 days	50 days	2–3 mo
Thyroid hormones	tT4 ↑ fT4 ↓ tT3 ↓ fT3 ↑ rT3 ↓	No change in TSH T3 ↓ rT3 ↑	tT4 ↓ T3 ↓ fT3 ↓ rT3 ↑	T3 ↓ T4 ↓	T3 ↓ T4 ↓ rT3 ↓	T3 ↓	T3 ↓ T4 ↑	All forms maintained or increased with fasting duration, except, tT4 ↓	Exhibited a peak in T3 levels, whereas T4 levels declined substantially	T3 ↑ T4 ↑ rT3 ↓
Plasma glucose	↓ (60 hours, 4–6 days fasting)	↓ (15–36 days fasting)	↓	Did not change	Delayed glucose response to exogenous insulin	Did not change	↑	↑	↑	↓
Plasma insulin	↓ (5 days)	↓ (15–36 days fasting)	↓	↓	Delayed insulin responses to external stimulation during hibernation	↓	↓	↓	did not change	↓
Other hormones/metabolites	TSH ↓ Blunted TSH response to TRH	Plasma glucagon ↑ Growth hormone ↑ Triglycerides ↑		Plasma ghrelin ↑ Plasma glucagon ↓		Plasma triglyceride ↓	Plasma triglyceride ↑ Glycerol ↑	Plasma glucagon ↓ Plasma leptin ↓	Plasma glucagon ↑	Adipose DAG ↑ Adiponectin ↑ Nonesterified fatty acid ↑
Refs.	6, 20, 23, 62, 118	7, 62	46, 51, 94, 66		8, 83, 104, 113, 122	35	91	128	26	80, 81, 100, 101, 127, 130–133

A summary of human, rat, and comparative animal studies* assessed, demonstrating thyroid, glucose, insulin, and other metabolites/hormones response to fasting. TRH, thyrotropin-releasing hormone; TSH, thyroid stimulating hormone; T4, thyroxine; tT4, total thyroxine; fT4, free thyroxine; T3, triiodothyronine; tT3, total triiodothyronine; fT3, free triiodothyronine; rT3, reverse triiodothyronine; DAG, diacylglycerol. *Data for king penguins is added for comparative purposes to provide some insight on evolutionary changes.

exogenous (11, 16, 22, 71, 72, 76). *Phase I* is followed by a comparatively longer *phase II* (generally the longest), in which lipid stores are mobilized to near depletion and lean tissue is spared. During *phase II*, metabolic rates are continually depressed, and the utilized glucose is now a product of primarily gluconeogenesis (11, 16, 71, 72, 76). In *phase III*, the organism transitions from lipid catabolism to protein catabolism, a phase that can be averted if feeding resumes. Body mass loss is accelerated in *phase III* (105). *Phase III* is often characterized by irreversible protein catabolism, leading to death due to functional degeneration of cells if feeding is not promptly restored (11, 16, 71, 72, 76). The transitions between phases are associated with dynamic shifts in cellular metabolism and ultimately respiratory quotient (RQ). However, in naturally adapted animals, although the metabolic shifts may be extremely dynamic at a cellular level, these shifts do not usually translate into profound changes in RQ or the development of ketoacidosis. Hence, a principal distinction between starvation and adaptive fasting is the maintenance of a relatively stable RQ (usually closer to 0.70), independent of ketoacidosis and cachexia (55, 56, 114). Additionally, given that metabolic depression is a key component to adaptive fasting (in most mammals) that extends endogenous fuel supplies, endocrine modulation of metabolism must also be tightly regulated. Cellular metabolism is regulated primarily by thyroid hormones (TH), which are decreased across almost all mammals during times of either short or especially prolonged food deprivation or starvation (6, 16, 21, 22, 33, 93, 94, 98, 110, 138). Given that TH are the major endocrine modulators of metabolic rates, having profound effects

on substrate metabolism, oxygen consumption, and subsequently RQ, the next section will focus on and examine thyroid responses to fasting (49).

Thyroid Hormone-Driven Cellular Metabolism and Fasting

The secretion of TH from the thyroid gland is regulated by the hypothalamic-pituitary-thyroid (HPT) axis (58, 89). Stimulation of the gland by the pituitary-derived hormone, thyroid stimulating hormone (TSH), results in the secretion of both triiodothyronine (T3) and thyroxine (T4) into circulation, which enter the cell membrane through transporters such as alpha-v beta-3 and monocarboxylate transporter 8 (MCT8) (41, 116). Until recently, T4 was considered a prohormone to the biologically active T3, given the inability of T4 to bind its nuclear receptor and induce TH-mediated genomic action (32, 61). This canonical, classical genomic TH pathway requires the deiodination of T4 by either deiodinase type 1 (DI1) or 2 (DI2) (67). Their capacity to deiodinate T4 in enzyme-specific manners, i.e., removal of iodine atoms from phenolic (outer) or tyrosil (inner) rings, the rate of deiodination, or the enzymes activity, offers a crucial regulatory step in TH-mediated events that is likely as important as the regulation of TH secretion. For example, with the capacity to remove iodine moieties from either of the phenolic or tyrosil rings, DI1 has the capacity to convert T4 into T3 (phenolic deiodination) and reverse T3 (rT3) (tyrosil deiodination). Reverse T3 is the inactive form of T3, primarily formed by deiodination by DI3, and lacks the capacity to properly bind nuclear TH receptors. Unlike DI1, DI2 and DI3 can

only remove iodine moieties from either the outer or the inner rings, respectively, making DI2 primarily responsible for activation of TH, and DI3 primarily responsible for suppression of cellular metabolism (14, 27, 54, 85). Once converted to T3, this biologically active form of TH binds its tissue-specific nuclear receptor, which then activates the thyroid response element (TRE) and drives the transcription of genes associated with substrate metabolism via the recruitment of co-activator complexes (43, 126). However, recently, it has become more evident that T4 has a potent, non-genomic capacity to drive metabolism, which may be much more efficient because the mechanism is independent of binding of the nuclear receptor (32, 61).

A thorough review of the literature reveals that the typical response to fasting is suppression of TH-mediated cellular events. This response is well documented in not only humans and rodents but also in natural models of adaptive fasting. For example, in humans, TSH is suppressed with fasting duration, resulting in reduced circulating levels of TH (6, 7, 21, 118). Moreover, DI activity and receptor availability are also both downregulated in humans during fasting (4, 97). In rodents, caloric restriction also depressed TH levels (51, 66), and complete fasting decreased DI1 activity coupled

with increased circulating levels of rT3 (121). In hibernating black bears, levels of free T4 (fT4) and T3 (fT3) decreased with fasting duration (122). However, in hibernating little brown bats (*Myotis lucifugus*), total T4 (tT4) levels increased during hibernation with notably high levels observed during arousal (26). This paradoxical increase in tT4 was suggested to be physiologically regulating or contributing to important sex-related changes rather than increasing cellular metabolism (30). However, in female sheath-tailed bats (*Taphozous longimanus*), the thyroid gland remained inactive during early to mid-winter dormancy, becoming active during late winter dormancy, which was coupled with the breeding season (124). Last, in hibernating ground squirrels (*Spermophilus richardsoni*), circulating levels of TH increased, absent the availability of hepatic nuclear receptors, rendering the levels cryptically hyperthyroid (93, 94). Despite the variability in the fasting-induced responses of TH and its cellular-associated actions, the majority of these data suggest that food deprivation suppresses TH-mediated cellular events or renders target-tissues unavailable to TH-mediated actions (FIGURE 1).

Intriguingly, unlike most fasting mammals, northern elephant seal pups do not decrease cellular TH-mediated events (80, 81, 101). Their

FIGURE 1. Schematic representation of cellular, thyroid hormone mediate activity

Schematic representation of cellular, thyroid hormone mediate activity in humans (A), squirrels (B), rodents (C), and elephant seals (D). αvβ3, Alpha-v beta-3; MCT8, monocarboxylate transporter 8; T4, thyroxine; T3, triiodothyronine; rT3, reverse triiodothyronine; DI1, deiodinase type 1; DI2, deiodinase type 2; DI3, deiodinase type 3; ER, endoplasmic reticulum; TRE, thyroid hormone response element; RXR, retinoid X receptor; TRβ1, thyroid hormone receptor beta 1; Co-Act, co-activator complexes; HAT, histone acetyl transferase; NCo-R, nuclear co-repressor complexes; HDAC, histone deacetylase activity.

unique 2- to 3-mo fast is coupled with paradoxical changes in thyroidal function (80, 81, 101, 127, 129–133). Although their fast is characterized by modest increases in circulating levels of T4 and T3, recently we showed that these modest changes masked the substantial increases in TH secretion due to increased cellular metabolism of the circulating levels with fasting duration (80, 81) (FIGURE 1).

Thyroid Status and Insulin Resistance

Another endocrine response/consequence of food deprivation is the onset of insulin resistance that spares glucose for glucose-dependent tissues such as the central nervous system (28, 42, 48, 52, 53, 131, 133). Fasting in humans is associated with a drop in plasma glucose, a drop that is sustained for up to 33 days of fasting, and decreased plasma insulin; a similar response is seen in rats (23, 46, 62). Interestingly, derangements in circulating levels of TH are associated with insulin resistance. Seminal studies have highlighted the contribution of thyroid hormone-associated derangements to the incidence of insulin resistance, wherein a partial thyroidectomy was associated with improvements in insulin resistance and restored glucose tolerance in diabetic subjects with hyperthyroidism (9, 57, 64, 70). It is therefore not surprising that thyroid disease and diabetes mellitus are the two most common endocrine-related derangements treated concurrently in medical practice today (135). Thus it has become increasingly evident that TH and insulin signaling are related, wherein an aberration in one can lead to dysregulation in the other (39). Moreover, the contributions of TH to insulin signaling and glucose metabolism, albeit poorly defined to date, are evident even in euthyroid human subjects in which low levels of T4 within the normal range are correlated with increased prevalence of insulin resistance (68, 99, 107–109).

Dysregulation of carbohydrate metabolism and thyroidal derangements are strongly correlated. This is evident from studies that show that the severity of the metabolic disorder is linearly proportional to the thyroidal derangement, and from other studies that suggest that the effects of T3 on glucose metabolism are as substantial as those of insulin (17, 75, 81). TH can directly and indirectly regulate insulin secretion either by reducing glucose-induced insulin secretion or by reducing β -cell responsiveness that results from increases in cell mass in hypo- and hyperthyroidisms, respectively (87, 120). It is worth noting the importance of tissue cross talk in the development of insulin resistance, given that proper insulin signaling is

restored when cross talk between tissue types is removed. For example, muscle cells taken from insulin-resistant subjects respond normally to insulin, which is not the case when myocytes and adipocytes are co-cultured (106). Given that normal glucose regulation can be disrupted by either an excess or a diminished amount of TH, the next two sections will focus on the contributions of the two to disrupting normal glucose utilization and insulin signaling.

Hyperthyroidism and Insulin Resistance

The physiological role of TH in increasing the rate of metabolism has been well established (33, 74, 84, 111). In a hyperthyroid state, glucose depletion increases substantially to counteract the loss in energy, increasing the demand for insulin in peripheral tissues, which is itself driven by increases in insulin-stimulated glucose oxidation rates. This increase in utilization rate eventually leads to a decrease in hepatic insulin sensitivity (25, 37). Hyperthyroidism is also associated with increased venous blood flow associated with increased absorption of glucose from the GI tract, which leads to hyperglycemia and the subsequent increase in glucose-stimulated insulin secretion (20). Impaired glucose tolerance, in hyperthyroid subjects, is therefore largely an effect of increased glucose turnover as a result of increased GI glucose absorption, which also leads to postabsorptive hyperglycemia, increased hepatic glucose output, as well as increased fasting and postprandial insulin (20). Additionally, hyperthyroid subjects are more prone to increased ketoacidosis resulting from excessive thyroid-stimulated lipolysis (13). The effects of excess TH are quite variable in different tissues, exerting both insulin agonistic and antagonistic actions in a tissue-specific manner. In normal subjects, this occurs in physiological balance, maintaining normal glucose metabolism. Hyperthyroidism is associated with increased hepatic gluconeogenesis through either the canonical transcription-mediated TH pathway involving the gluconeogenic-driving proteins and glucose transporters or as a consequence of secondary influences on hepatocytes, thereby decreasing hepatic insulin sensitivity (88, 136). Hyperthyroid-induced hepatic insulin resistance is mediated through a combination of receptor-mediated gene transcription actions, increased glucose transporter 2, increased glucose efflux, and increased plasma free fatty acids (88, 112, 136). More specifically, hyperthyroidism leads to impaired glucose tolerance, which has been observed following oral glucose loading in hyperthyroid subjects (88, 112, 136). Studies suggest that this is caused by abnormal glucose metabolism in peripheral tissues

such as fat and muscle, and abnormal hepatic glucose metabolism, which TH directly contribute to (8, 112, 136). Abnormal increases in glucose efflux and gluconeogenesis contribute to impaired glucose tolerance (8, 112, 136). Gluconeogenesis in the liver leads to quick glucose transport across the hepatocyte's plasma membrane, and this efflux of glucose across the hepatocyte plasma membrane is facilitated by transporters such as glucose transporter 2 (GLUT2), highlighting the transporter's role in the impairment of hepatic insulin action, the expression of which is also regulated by TH genomic action (8, 112, 136). In peripheral tissues, such as muscle and adipose, hyperthyroidism increases glucose utilization by increasing gluconeogenesis via hepatic glucose production during fasting and through the Cori cycle in postprandial states (87, 115). In adipose, hyperthyroidism has been associated with both increased and decreased insulin sensitivity (87). Increased concentrations of adipokines, including interleukin 6 and tumor necrosis factor alpha, may promote insulin resistance in peripheral tissues in hyperthyroidism (86). Hyperthyroidism also increased glycolysis in muscle, with a concomitant increase in peripheral lactate, which becomes a substrate for hepatic glucose production. Furthermore, the severity of the dysregulation in TH-mediated events increases with age. For example, mice lacking a mitochondrial T3 receptor are more susceptible to glucose intolerance and become more insulin resistant with age, suggesting that organelle-specific perturbations contribute to the development of insulin resistance, especially as it pertains to pancreatic β -cell dysfunction (12). Peripheral glucose utilization is increased in hyperthyroid states, as measured by euglycaemic hyperinsulinaemic clamp (36). Additionally, insulin resistance associated with hyperthyroidism likely involves changes in other hormones. Changes in overall energy homeostasis, as indicated by hypoghrelinemia and increased glucagon-to-ghrelin ratios, have been implicated in the development of insulin resistance in hyperthyroidism (2). Additionally, adiponectin, an important adipokine that increases insulin sensitivity, is elevated in hyperthyroidism (2, 69) (FIGURE 2).

Hypothyroidism and Insulin Resistance

Hypothyroidism has also been associated with several key peripheral effects on insulin secretion and glucose metabolism. In contrast to hyperthyroidism, hypothyroidism reduces glucose absorption from the GI tract (31). It is also associated with an almost complete cessation of hepatic glucose production and an increase in insulin levels (3).

Homeostasis model assessment insulin resistance (HOMA-IR) values were higher in subjects with subclinical hypothyroidism compared with euthyroid subjects (134), suggesting that reduced TH levels contribute to insulin resistance. Moreover, TSH was positively correlated with insulin and HOMA-IR values, whereas ft_4 and ft_3 were inversely correlated with insulin and HOMA-IR (134). Although these relationships may be difficult to reconcile in the face of the data on associations between hyperthyroidism and insulin resistance, they do suggest that alterations in cellular TH-associated events (i.e., deiodination) have the potential to contribute to the development of insulin resistance and glucose disposal (134). Several other studies have reported that increases in TSH levels are associated with insulin resistance and clearly demonstrate that hypothyroidism is linked to increases in insulin resistance (9, 134). Even modest increases in TSH are associated with subclinical hypothyroidism, and are correlated with insulin resistance and hyperlipidemia (15, 59, 68, 77). TSH has the capacity to directly induce adipogenesis, and PPAR γ receptor agonism can potently stimulate TSH receptor expression and adipogenesis (125). TSH may also regulate the expression of adipokines through a TSHr-mediated process (117) (FIGURE 2).

Fasting-Adapted Mammals as Natural Models for Human Disease

Perhaps the most confounding aspect of elucidating the contributions of TH to insulin signaling and resistance is that different types of TH-associated aberrations have profoundly different outcomes. Even more perplexing is that some studies have found no correlation between thyroid hormone status and insulin sensitivity (38, 50, 103). In

FIGURE 2. Effects of thyroid hormones (hyper- and hypothyroidism) on hepatic glucose metabolism in euthyroid, hypothyroid, and hyperthyroid conditions

addition, the choice of model used, which is often-times either a genetically or pharmaceutically modified animal that is engineered to induce either hypo- or hyperthyroidism, can be a confounding variable that contributes to incongruent data among studies. For example, a transgenic mouse expressing the human thyroid-stimulating Ab (TSAb) lacks the lymphocyte aggregates characteristic of Graves's disease, making immunological studies associated with TH challenging (63). Other models include immunization with specific expression vectors or adenovirus, which may not be possible in many naturally existing animal models with a unique physiology (92). Therefore, studies in animals that have naturally evolved robust physiological mechanisms to tolerate extreme nutritional conditions, such as protracted periods of food and water deprivation, may provide unique opportunities to gain insight into the relationships between TH and insulin signaling. Although we recognize that much work remains to better elucidate the relationships between TH and insulin signaling, sufficient data now exist from animals naturally adapted to fasting to allow for some intriguing insights.

In raccoon dogs (*Nyctereutes procyonoides*), circulating levels of T4 decreased after 4 wk of fasting, with levels increasing slightly after 8 wk, but not nearly back to baseline levels (95). Circulating levels of T3 also decreased after 4 wk of fasting and continued to fall after 8 wk, suggesting that the slight increases in T4 may have been the result of reduced DI1 or DI2, decreasing the potential for genomic TH-mediated effects (95). In these animals, plasma insulin levels decreased over 50% after 8 wk of fasting, indicating that fasting induced reciprocal changes in TH and insulin (95). The decreases in TH and insulin may be key adaptations to allow other factors or hormones to increase triglyceride hydrolysis while sparing lean tissue (decreased protein catabolism) (90). American black bears (*Ursus americanus*) hibernate between December and April, abstaining from both food and water intake (83). Injecting hibernating bears with insulin and glucagon, separately, showed that insulin clearance was delayed and glucose levels dropped in response to exogenous insulin; glucagon responses to exogenous insulin were also delayed (104). In response to exogenous glucagon, clearance of glucagon and return to baseline levels were delayed. Similarly, insulin clearance in response to exogenous glucagon was also suppressed during hibernation, delaying the return to baseline values (104). Furthermore, the corticosteroid response was blunted in response to exogenous glucagon administration compared with the insulin infusion. Overall, a decrease in plasma glucose coupled with delayed insulin responses to external

stimulation during hibernation indicated increased lipolysis and a metabolic shift toward lipid mobilization (104). Additionally, hibernation-associated food deprivation induced hypothyroidism in bears, despite the fact that examination of the thyroid gland from a bear after 2 mo of hibernation, revealed normal histology (113, 122), suggesting that the hypothyroidism had no aberrant effects on the gland. Paradoxically, levels of rT3 also decreased during hibernation, suggesting that the hypothyroidism associated with hibernation was likely not a function of rT3 antagonism of the TH receptor (8). Interestingly, the hypothyroidism observed in hibernating black bears and its associated transient insulin resistance, assessed by combined glucose and insulin tolerance tests, occurred whether the animal hibernated or not. This intriguing finding was demonstrated in a study where captive, non-hibernating bears retained similar physiological responses to the winter season as their wild and hibernating counterparts (83).

In captive gray wolves (*Canis lupus*), T3 and plasma insulin levels decreased during a 10-day fasting period, although glucose levels were not affected (35). Comparatively, in fed wolves, increased plasma insulin levels were likely the result of an influx of glucose from absorption to portal circulation (35). Moreover, triglyceride levels decreased in fasted wolves, suggesting that TG hydrolysis was increased and may partially explain the maintenance of plasma glucose levels. Similarly, T3 and plasma insulin levels also decreased in fasted blue foxes (*Alopex lagopus*); however, T4 levels remained elevated, if not maintained, with a 22-day fast (91). Additionally, the initial, acute (first 48 h) period was characterized by increases in plasma glucose in the presence of decreasing insulin. This same phenomenon has been observed in the domestic dog, where the rate of liver glycogen degradation is the most dramatic at the onset of food deprivation, specifically the first 48 h (34). Following the depletion of glycogen storage, gluconeogenesis became the main metabolic support, which caused hypoglycemia in the blue foxes during some fasting phases. De novo glucose synthesis was likely a result of decreasing plasma insulin, coupled with increases in plasma glucagon.

In sub-Antarctic fur seal (*Arctocephalus tropialis*) pups, plasma TH levels were either maintained or increased with fasting duration, with exception of tT4, which decreased (128). The maintenance of fT4, fT3, and tT3 with fasting in seal pups may indicate reduced deiodinase activity coupled with a lack of any effect on receptor availability. Thus decreases in deiodinase activity/content and receptor binding could account for reduced hormone clearance from circulation, resulting in either elevated or maintained circulating levels. A

drop in insulin levels early in the fast were associated with a 20% increase in glucose levels, as would be expected, but in so doing over a prolonged period, it would contribute to protein sparing and promoting lipolysis (128).

King penguins (*Aptenodytes patagonicus*) undergo unique hormonal and metabolic changes during breeding, which is also associated with prolonged food deprivation. At the beginning of their fast, king penguins exhibited a peak in T3 levels while T4 levels declined substantially (26). However, by the end of *phase II*, and during *phase III* of the starvation paradigm, T3 decreased to undetectable levels. During *phase II*, gluconeogenesis decreased, marking the metabolic switch to lipid oxidation to sustain energy expenditure and spare lean tissue (26). During the overall fast, plasma insulin levels did not change but were characterized by increased plasma glucagon and decreased plasma T3 and T4, suggesting that the protein sparing phase in King penguins necessitates low insulin and TH plasma levels. During *phase III*, characterized by a shift into protein utilization, fasting was associated with increased plasma corticosterone and glucagon, likely enabling gluconeogenesis from amino acids (26). Although king penguins are birds, the changes to prolonged fasting have a striking resemblance to that seen in mammals adapted to fasting; the phases of fasting and their associated hormonal and metabolic changes are similar to those found in most mammals and birds (26, 44, 45).

Northern elephant seal (*Mirounga angustirostris*) pups undergo a 2- to 3-mo post-weaning fast (18), characterized by a perplexing suite of physiological events (80, 81, 100, 101, 127, 129–133). Over the course of their postweaning fast, pups develop adipose-specific insulin resistance associated with an increase in TH production, leading to increased cellular TH-mediated responses (79, 82, 129, 130, 132, 133). In response to a TSH infusion, both T4 and T3 increased in late-fasted pups. These increases occurred while muscle DI1 and DI2 expressions increased, suggesting that the fast enhances cellular TH-mediated events likely to support the animal's energetic needs as the fast progresses. More recently, our group has shown that DI3 expression levels are regulated epigenetically, since methylation increased in muscle much faster than in adipose (78, 79, 82). These data suggest a strong inhibition via epigenetic modification of the suppressive mechanisms of cellular metabolism to permissively increase TH-mediated cellular events. These, and likely other, epigenetic effects may constitute a suite of physiological adaptations that allow seals to tolerate and thrive during protracted periods of food deprivation. Associated with these changes in TH signaling during

prolonged fasting were decreases in insulin and adiponectin, which combined would desensitize insulin signaling. The prolonged fast in elephant seal pups is also characterized by relatively high blood glucose levels, increased nonesterified fatty acid, adipose diacylglycerol (DAG) accumulation, and impaired adipose insulin signaling (130, 133). Collectively, the thyroidal status of fasting seals would be considered euthyroid, since the cellular capacity is enhanced with fasting duration despite the presence of tissue-specific insulin resistance, suggesting that the contribution of TH and insulin signaling to the adaptation to prolonged fasting may be unique in these animals.

Insights Into Thyroidal Modulation of Insulin Resistance From Fasting-Adapted Mammals

Examining the specific thyroidal changes associated with normal responses to physiological adaptations such as fasting is a useful tool in the assessment of how this endocrine system contributes to changes in substrate metabolism and insulin signaling. Careful study of several unique fasting-adapted mammals has provided valuable information that mitigates undesirable side-effects that can complicate interpretation of results gained from pathophysiological models. When human subjects become insulin resistant, it is normally the result of impaired insulin signaling in muscle given that muscle is the largest glucose sink in the body. Yet, northern elephant seal pups, which have exceptionally large adipose stores [~45% of body mass (96, 100)] develop adipose-specific insulin resistance while fasting, whereas muscle remains insulin sensitive (129, 131–133). Studies with northern elephant seals suggest that the fasting-induced increase in adipose DAG accumulation may be a contributing factor in the development of cellular insulin resistance (130), although the signal that drives DAG accumulation in these animals is still under investigation. Perfusion of livers of hypothyroid mice with T4 increased hepatocyte DAG accumulation, suggesting that T4 drives DAG accumulation (60). Additionally, T4 was shown to non-genomically drive DAG accumulation in hepatocytes via activation of phospholipase C, phospholipase D, and protein kinase C (61), suggesting that the link between thyroid hormones and insulin resistance may be hepatic (or other tissue) via DAG accumulation. Another clue may lie in the processes that generate fT4, since a number of fasting-adapted animals display an increase in fT4 in response to fasting, including hibernation (93, 94, 102). In addition, another intriguing mechanism may be signaling through the peripheral TSH receptor, which has

been reported in many non-thyroid tissues, including adipose (1, 10). Activation of adipose TSHr led to DAG accumulation and increased activity and concentration of pertinent kinases and second messengers (24, 139). Although the expression of TSHr in muscle is controversial (19, 119), confirmation of its presence would be significant because of the importance of muscle to glucose regulation and its susceptibility to the development of insulin resistance. In addition to driving DAG accumulation, TSH correlated well with body mass index values and positively correlated with HOMA-IR values (134). This suggests that TSH and its peripheral receptor may be novel contributors to fasting-induced adaptations in TH- and insulin-mediated signaling. Because TSH levels are not consistently decreased in the fasting-adapted mammals that have been examined (e.g., black bears and northern elephant seals), and the thyroid gland appears to be resistant to the consequences of hibernation-associated fasting (113), TSH could be an important contributing factor in the evolution of adaptive fasting.

Additionally, the transitions among the phases of substrate utilization are associated with dynamic shifts in cellular metabolism and RQ. However, in naturally adapted animals, although the metabolic shifts may be extremely dynamic at a cellular level, these shifts do not usually translate into profound changes in RQ, as demonstrated by the work in the northern elephant seal (73, 79). The present review has highlighted incongruences in the data and expounded on the variability in the responses of TH-mediated signaling and its potential contribution to insulin resistance among an array of mammals. However, further investigation into the relationships between TH and insulin signaling as it relates to insulin resistance using non-traditional animal models may prove highly beneficial. Elephant seals, for example, can provide an intriguing model to better understand the TH-mediated reliance on lipid metabolism that is not otherwise present in morbidly obese humans that may elucidate potential therapeutic targets of thyroid hormones for human obesity. Nonetheless, the full complement of factors that contribute to the coordinated regulation of thyroid hormone and insulin signaling, including those that modulate intermittent periods of reversible insulin resistance, remains to be discovered. Undoubtedly, the study of animals that have naturally evolved robust physiological mechanisms that allow them to tolerate and thrive in extreme environmental and nutritional conditions should contribute significantly in this discovery process. ■

No conflicts of interest, financial or otherwise, are declared by the author(s).

Author contributions: B.M. performed experiments; B.M. analyzed data; B.M. and R.M.O. interpreted results of experiments; B.M. prepared figures; B.M. and R.M.O. drafted manuscript; B.M. and R.M.O. edited and revised manuscript; B.M. and R.M.O. approved final version of manuscript.

References

1. Sorisky A, Bell A, Gagnon A. TSH Receptor in Adipose Cells. *Horm Metab Res* 32: 468–474, 2000.
2. Ağbaht K, Erdogan MF, Emral R, Baskal N, Güllü S. Circulating glucagon to ghrelin ratio as a determinant of insulin resistance in hyperthyroidism. *Endocrine* 45: 106–113, 2013.
3. Althausen TL, Stockholm M. The influence of the thyroid gland on absorption in the digestive tract. *Am J Physiol* 123: 577–588, 1938.
4. Araujo RL, de Andrade BM, de Figueiredo ÁSP, da Silva ML, Marassi MP, dos Santos Pereira V, Bouskela E, Carvalho DP. Low replacement doses of thyroxine during food restriction restores type 1 deiodinase activity in rats and promotes body protein loss. *J Endocrinol* 198: 119–125, 2008.
5. Atkinson S, Arnould JPY, Mashburn KL. Plasma cortisol and thyroid hormone concentrations in pre-weaning Australian fur seal pups. *Gen Comp Endocrinol* 172: 277–281, 2011.
6. Azizi F. Effect of dietary composition on fasting-induced changes in serum thyroid hormones and thyrotropin. *Metabolism* 27: 935–942, 1978.
7. Azizi F. Islamic fasting and thyroid hormones. *Intl J Endocrinol Metab* 13: e29248, 2015.
8. Azizi FMJ, Howard D, Nelson RA. Effect of winter sleep on pituitary-thyroid axis in American black bear. *Am J Physiol Endocrinol Metab* 237: E227–E230, 1979.
9. Ujwal Upadya B, Suma MN, Srinath KM, Prashant A, Doddamani P, Shilpa SV. Effect of Insulin Resistance in Assessing the Clinical Outcome of Clinical and Subclinical Hypothyroid Patients. *J Clin Diagn Res* 9: OC01–OC04, 2015.
10. Balzan S, Nicolini G, Forini F, Boni G, Del Carratore R, Nicolini A, Carpi A, Iervasi G. Presence of a functional TSH receptor on human erythrocytes. *Biomed Pharmacother* 61: 463–467, 2007.
11. Benedict FG, Leer C. *Lipogenesis in the Animal Body, With Special Reference to the Physiology of the Goose*. Washington, DC: Washington Carnegie Inst., 1937.
12. Bertrand C, Blanchet E, Pessemesse L, Annicotte JS, Feillet-Coudray C, Chabi B, Levin J, Fajas L, Cabello G, Wrutniak-Cabello C, Casas F. Mice lacking the p43 mitochondrial T3 receptor become glucose intolerant and insulin resistant during aging. *PLoS One* 8: e75111, 2013.
13. Beylot M. Regulation of in vivo ketogenesis: role of free fatty acids and control by epinephrine, thyroid hormones, insulin and glucagon. *Diabetes Metab* 22: 299–304, 1996.
14. Bianco AC, Kim BW. Deiodinases: implications of the local control of thyroid hormone action. *J Clin Invest* 116: 2571–2579, 2006.
15. Bilgin H, Pirgon Ö. Thyroid function in obese children with non-alcoholic fatty liver disease. *J Clin Res Pediatr Endocrinol* 6: 152–157, 2014.
16. Boismenu C, Gauthier G, Larochelle J. Physiology of prolonged fasting in greater snow geese (*Chen caerulescens atlantica*). *Auk* 109: 511–521, 1992.
17. Bollinger SS, Weltman NY, Gerdes AM, Schlenker EH. T3 supplementation affects ventilatory timing and glucose levels in Type 2 diabetes mellitus model. *Respir Physiol Neurobiol* 205: 92–98, 2015.
18. Bonner W. *The Natural History of Seals*. New York: Facts On File, 1990.
19. Boschi A, Daumerie C, Spiritus M, Beguin C, Senou M, Yuksel D, Duplcy M, Costagliola S, Ludgate M, Many MC. Quantification of cells expressing the thyrotropin receptor in extraocular muscles in thyroid associated orbitopathy. *Br J Ophthalmol* 89: 724–729, 2005.

20. Brenta G. Why can insulin resistance be a natural consequence of thyroid dysfunction? *J Thyroid Res* 2011: 152850, 2011.
21. Burman KD, Smallridge RC, Osburne R, Dimond RC, Whorton NE, Kesler P, Wartofsky L. Nature of suppressed TSH secretion during undernutrition: effect of fasting and refeeding on TSH responses to prolonged TRH infusions. *Metabolism* 29: 46–52, 1980.
22. Cahill GF, Herrera MG, Morgan AP, Soeldner JS, Steinke J, Levy PL, Reichard GA, Kipnis DM. Hormone-fuel interrelationships during fasting. *J Clin Invest* 45: 1751–1769, 1966.
23. Carlson MG, Snead WL, Campbell PJ. Fuel and energy metabolism in fasting humans. *Am J Clin Nutr* 60: 29–36, 1994.
24. Cass LA, Meinkoth JL. Differential effects of cyclic adenosine 3',5'-monophosphate on p70 ribosomal S6 kinase. *Endocrinology* 139: 1991–1998, 1998.
25. Cavallo-Perin P, Bruno A, Boine L, Cassader M, Lenti G, Pagano G. Insulin resistance in Graves' disease: a quantitative in-vivo evaluation. *Eur J Clin Invest* 18: 607–613, 1988.
26. Cherel Y, Robin JP, Walch O, Karmann H, Netchitailo P, Le Maho Y. Fasting in king penguin. I. Hormonal and metabolic changes during breeding. *Am J Physiol Regul Integr Comp Physiol* 254: R170–R177, 1988.
27. Ciavardelli D, Bellomo M, Crescimanno C, Vella V. Type 3 deiodinase: role in cancer growth, stemness, and metabolism. *Front Endocrinol* 5: 215, 2014.
28. van der Crabben SN, Allick G, Ackermans MT, Ender E, Romijn JA, Sauerwein HP. Prolonged fasting induces peripheral insulin resistance, which is not ameliorated by high-dose salicylate. *J Clin Endocrinol Metab* 93: 638–641, 2008.
29. Crocker DE, Champagne CD, Fowler MA, Houser DS. Adiposity and fat metabolism in lactating and fasting northern elephant seals. *Adv Nutr Res* 5: 57–64, 2014.
30. Damassa DA, Gustafson AW, Kwiecinski GG, Gagin GA. Seasonal influences on the control of plasma sex hormone-binding globulin by T4 in male little brown bats. *Am J Physiol Regul Integr Comp Physiol* 268: R1303–R1309, 1995.
31. Daher R, Yazbeck T, Jaoude JB, Abboud B. Consequences of dysthyroidism on the digestive tract and viscera. *World J Gastroenterol* 15: 2834–2838, 2009.
32. Davis PJ, Leonard JL, Davis FB. Mechanisms of nongenomic actions of thyroid hormone. *Front Neuroendocrinol* 29: 211–218, 2008.
33. Dayan CM, Panicker V. Novel insights into thyroid hormones from the study of common genetic variation. *Nat Rev Endocrinol* 5: 211–218, 2009.
34. de Bruijne JJ, de Koster P. Glycogenolysis in the fasting dog. *Comp Biochem Physiol* 75: 553–555, 1983.
35. Del Giudice GD, Seal US, Mech LD. Effects of feeding and fasting on wolf blood and urine characteristics. *J Wildlife Management* 51: 1–10, 1987.
36. Dimitriadis G, Baker B, Marsh H, Mandarino L, Rizza R, Bergman R, Haymond M, Gerich J. Effect of thyroid hormone excess on action, secretion, and metabolism of insulin in humans. *Am J Physiol Endocrinol Metab* 248: E593–E601, 1985.
37. Dimitriadis G, Baker B, Marsh H, Mandarino L, Rizza R, Bergman R, Haymond M, Gerich J. Effect of thyroid hormone excess on action, secretion, and metabolism of insulin in humans. *Am J Physiol Endocrinol Metab* 248: E593–E601, 1985.
38. Dulaurent S, Gaulier JM, Marquet P, Lachâtre G. Traitement des demandes de dosages de pesticides dans les milieux biologiques: quelques problèmes fréquemment rencontrés. *Acta Clin Belg* 61: 69–74, 2006.
39. Fernández-Real JM, López-Bermejo A, Castro A, Casamitjana R, Ricart W. Thyroid function is intrinsically linked to insulin sensitivity and endothelium-dependent vasodilation in healthy euthyroid subjects. *J Clin Endocrinol Metab* 91: 3337–3343, 2006.
40. Florant G, Healy J. The regulation of food intake in mammalian hibernators: a review. *J Comp Physiol B* 182: 451–467, 2012.
41. Fonseca TL, Correa-Medina M, Campos MPO, Wittmann G, Werneck-de-Castro J.P, Arrojo e Drigo R, Mora-Garzon M, Ueta CB, Caicedo A, Fekete C, Gereben B, Lechan RM, Bianco AC. Coordination of hypothalamic and pituitary T3 production regulates TSH expression. *J Clin Invest* 123: 1492–1500, 2013.
42. Freitas MB, Queiroz JF, Dias Gomes CI, Collares-Buzato CB, Barbosa HC, Boschero AC, Gonçalves CA, Pinheiro EC. Reduced insulin secretion and glucose intolerance are involved in the fasting susceptibility of common vampire bats. *Gen Comp Endocrinol* 183: 1–6, 2013.
43. Gauthier C, Chassande O, Plateroti M, Roux JP, Legrand C, Pain B, Rousset B, Weiss R, Trouillas J, Samarut J. Different functions for the thyroid hormone receptors TRα and TRβ in the control of thyroid hormone production and post-natal development. *EMBO J* 18: 623–631, 1999.
44. Goodman MNLP, Kaplan MM, Aoki TT, Young VR, Ruderman NB. Starvation in the rat. II. Effect of age and obesity on protein sparing and fuel metabolism. *Am J Physiol Endocrinol Metab* 239: E277–E286, 1980.
45. Goodman MN, McElaney MA, Ruderman NB. Adaptation to prolonged starvation in the rat: curtailment of skeletal muscle proteolysis. *Am J Physiol Endocrinol Metab* 241: E321–E327, 1981.
46. Grey NJ, Goldring S, Kipnis DM. The effect of fasting, diet, and actinomycin D on insulin secretion in the rat. *J Clin Invest* 49: 881–889, 1970.
47. Groscolas R, Cherel Y. How to molt while fasting in the cold: the metabolic and hormonal adaptations of emperor and king penguins. *Ornis Scand* 23: 328–334, 1992.
48. Hanssen MJW, Wiers R, Hoeks J, Gemmink A, Brans B, Mottaghy FM, Schrauwen P, van Marken Lichtenbelt WD. Glucose uptake in human brown adipose tissue is impaired upon fasting-induced insulin resistance. *Diabetologia* 58: 586–595, 2015.
49. Harper ME, Seifert EL. Thyroid hormone effects on mitochondrial energetics. *Thyroid* 18: 145–156, 2008.
50. Harris PE, Walker M, Clark F, Home PD, Albert KGMM. Forearm muscle metabolism in primary hypothyroidism. *Eur J Clin Invest* 23: 585–588, 1993.
51. Herlihy JT, Stacy C, Bertrand HA. Long-term food restriction depresses serum thyroid hormone concentrations in the rat. *Mech Ageing Dev* 53: 9–16, 1990.
52. Higashida K, Fujimoto E, Higuchi M, Terada S. Effects of alternate-day fasting on high-fat diet-induced insulin resistance in rat skeletal muscle. *Life Sci* 93: 208–213, 2013.
53. Hoeks J, van Herpen NA, Mensink M, Moonen-Kornips E, van Beurden D, Hesselink MKC, Schrauwen P. Prolonged fasting identifies skeletal muscle mitochondrial dysfunction as consequence rather than cause of human insulin resistance. *Diabetes* 59: 2117–2125, 2010.
54. Hong EG, Kim BW, Young Jung D, Hun Kim J, Yu T, Seixas Da Silva W, Friedline RH, Bianco SD, Seslar SP, Wakimoto H, Berul CI, Russell KS, Won Lee K, Larsen PR, Bianco AC, Kim JK. Cardiac expression of human type 2 iodothyronine deiodinase increases glucose metabolism and protects against doxorubicin-induced cardiac dysfunction in male mice. *Endocrinology* 154: 3937–3946, 2013.
55. Houser DS, Champagne CD, Crocker DE. A non-traditional model of the metabolic syndrome: the adaptive significance of insulin resistance in fasting-adapted seals. *Front Endocrinol* 4: 164, 2013.
56. Houser DS, Crocker DE, Tift MS, Champagne CD. Glucose oxidation and non-oxidative glucose disposal during prolonged fasts of the northern elephant seal pup (*Mirounga angustirostris*). *Am J Physiol Regul Integr Comp Physiol* 303: R562–R570, 2012.
57. Collier FA, Huggins CB. Effect of hyperthyroidism upon diabetes mellitus: striking improvement in diabetes mellitus from thyroidectomy. *Ann Surg* 86: 877–884, 1927.
58. Joseph-Bravo P, Jaimes-Hoy L, Uribe RM, Charli JL. 60 years of neuroendocrinology: TRH, the first hypophysiotropic releasing hormone isolated: control of the pituitary-thyroid axis. *J Endocrinol* 226: T85–T100, 2015.
59. Kapadia KB, Bhatt PA, Shah JS. Association between altered thyroid state and insulin resistance. *J Pharmacol Pharmacotherap* 3: 156–160, 2012.
60. Krasilnikova OA, Kavok NS, Babenko NA. A rapid effect of thyroxine on accumulation of diacylglycerols and on activation of protein kinase C in liver cells. *Biochemistry (Moscow)* 65: 1331–1336, 2000.
61. Kavok NS, Krasilnikova OA, Babenko NA. Thyroxine signal transduction in liver cells involves phospholipase C and phospholipase D activation. Genomic independent action of thyroid hormone. *BMC Cell Biol* 2: 5, 2001.
62. Kerndt PR, Naughton JL, Driscoll CE, Loxterkamp DA. Fasting: the history, pathophysiology and complications. *West J Med* 137: 379–399, 1982.
63. Kim-Saijo M, Akamizu T, Ikuta K, Iida Y, Ohmori K, Matsubara K, Matsuda Y, Suzuki M, Matsuda F, Nakao K. Generation of a transgenic animal model of hyperthyroid Graves' disease. *Eur J Immunol* 33: 2531–2538, 2003.
64. Kim KJ, Kim BY, Mok JO, Kim CH, Kang SK, Jung CH. Serum concentrations of ghrelin and leptin according to thyroid hormone condition, and their correlations with insulin resistance. *Endocrinol Metab* 30: 318–325, 2015.
65. Kirby VL, Ortiz CL. Hormones and fuel regulation in fasting elephant seals. In: *Elephant Seals: Population Ecology, Behavior, and Physiology*. Berkeley, CA: The Regents of the University of California, 1994.
66. Kmiec Z, Kotlarz G, Smiechowska B, Mysliwski A. Thyroid hormones homeostasis in rats refed after short-term and prolonged fasting. *J Endocrinol Invest* 19: 304–311, 1996.
67. Köhrle J. Local activation and inactivation of thyroid hormones: the deiodinase family. *Mol Cell Endocrinol* 151: 103–119, 1999.
68. Kouidhi S, Berhouma R, Ammar M, Rouissi K, Jarboui S, Clerget-Froidevaux MS, Seugnet I, Abid H, Bchir F, Demeneix B, Guissouma H, El-gaied AB. Relationship of thyroid function with obesity and Type 2 diabetes in euthyroid Tunisian subjects. *Endocr Res* 38: 15–23, 2013.
69. Pontikides N, Krassas GE. Basic endocrine products of adipose tissue in states of thyroid dysfunction. *Thyroid* 17: 421–431, 2007.

70. Krysiak R, Gilowska M, Szkróbka W, Okopień B. The effect of metformin on the hypothalamic-pituitary-thyroid axis in patients with type 2 diabetes and amiodarone-induced hypothyroidism. *Pharmacol Rep* 68: 490–494, 2016.
71. Le Maho Y, Vu Van Kha H, Koubi H, Dewasmes G, Girard J, Ferré P, Cagnard M. Body composition, energy expenditure, and plasma metabolites in long-term fasting geese. *Am J Physiol Endocrinol Metab* 241: E324–E354, 1981.
72. Le Maho Y, Vu Van Kha H, Koubi H, Dewasmes G, Girard J, Ferré P, Cagnard M. Metabolic adaptations to long-term fasting in Antarctic penguins and domestic geese. *J Therm Biol* 8: 91–96, 1983.
73. Lester CW, Costa DP. Water conservation in fasting northern elephant seals (*Mirounga angustirostris*). *J Exp Biol* 209: 4283–4294, 2006.
74. Leung FC, Taylor JE, Van Iderstine A. Effects of dietary thyroid hormones on growth, plasma T3 and T4, and growth hormone in normal and hypothyroid chickens. *Gen Comp Endocrinol* 59: 91–99, 1985.
75. Lin Y, Sun Z. Thyroid hormone potentiates insulin signaling and attenuates hyperglycemia and insulin resistance in a mouse model of type 2 diabetes. *Br J Pharmacol* 162: 597–610, 2011.
76. Lowell BB, Ruderman NB, Goodman MN. Regulation of myofibrillar protein degradation in rat skeletal muscle during brief and prolonged starvation. *Metabolism* 35: 1121–1127, 1986.
77. Luna-Vazquez F, Cruz-Lumbreras R, Rodríguez-Castelán J, Cervantes-Rodríguez M, Rodríguez-Antolín J, Arroyo-Helguera O, Castelán F, Martínez-Gómez M, Cuevas E. Association between the serum concentration of triiodothyronine with components of metabolic syndrome, cardiovascular risk, and diet in euthyroid postmenopausal women without and with metabolic syndrome. *Springer Plus* 3: 1–8, 2014.
78. Martínez B, Gemmell N, Crocker DE, Ortiz RM. Deiodinase type 3 methylation increases in response to thyroid stimulating hormone in a fasting adapted mammal. *FASEB J* 30: 760–728, 2016.
79. Martínez B, Soñanez-Organis JG, MacKenzie D, Crocker D, Ortiz R. Thyroid gland remains responsive to thyroid stimulating hormone with fasting duration. *FASEB J* 29: 686–6, 2015.
80. Martínez B, Soñanez-Organis JG, Vázquez-Medina JP, Viscarra JA, MacKenzie DS, Crocker DE, Ortiz RM. Prolonged food deprivation increases mRNA expression of deiodinase 1 and 2, and thyroid hormone receptor β -1 in a fasting-adapted mammal. *J Exp Biol* 216: 4647–4654, 2013.
81. Martínez B, Soñanez-Organis JG, Viscarra JA, Jaques JT, MacKenzie DS, Crocker DE, Ortiz RM. Glucose delays the insulin-induced increase in thyroid hormone-mediated signaling in adipose of prolonged-fasted elephant seal pups. *Am J Physiol Regul Integr Comp Physiol* 310: R502–R512, 2016.
82. Martínez B, Soñanez JG, Ortiz RM. Prolonged food deprivation increases mRNA and protein expression of deiodinase 1 & 2, and thyroid hormone receptor β -1 in a fasting-adapted mammal. *FASEB J* 27: 937–924, 2013.
83. McCain S, Ramsay E, Kirk C. The effects of hibernation and captivity on glucose metabolism and thyroid hormones in American black bear (*Ursus americanus*). *J Zoo Wildlife Med* 44: 324–332, 2013.
84. McCulloch AJ, Johnston DG, Baylis PH, Kendall-Taylor P, Clark F, Young ET, Alberti KG. Evidence that thyroid hormones regulate gluconeogenesis from glycerol in man. *Clin Endocrinol* 19: 67–76, 1983.
85. Medina MC, Molina J, Gadea Y, Fachado A, Murillo M, Simovic G, Pileggi A, Hernández A, Edlund H, Bianco AC. The thyroid hormone-inactivating type iii deiodinase is expressed in mouse and human β -cells and its targeted inactivation impairs insulin secretion. *Endocrinology* 152: 3717–3727, 2011.
86. Mitrou P, Boutati E, Lambadiari V, Tsegka A, Raptis AE, Tountas N, Economopoulos T, Raptis SA, Dimitriadis G. Insulin resistance in hyperthyroidism: the role of IL6 and TNF α . *Eur J Endocrinol* 162: 121–126, 2010.
87. Mitrou P, Raptis SA, Dimitriadis G. Insulin action in hyperthyroidism: a focus on muscle and adipose tissue. *Endocr Rev* 31: 663–679, 2010.
88. Mokuno T, Uchimura K, Hayashi R, Hayakawa N, Makino M, Nagata M, Kakizawa H, Sawai Y, Kotake M, Oda N, Nakai A, Nagasaka A, Itoh M. Glucose transporter 2 concentrations in hyper- and hypothyroid rat livers. *J Endocrinol* 160: 285–289, 1999.
89. Muller R, Liu YY, Brent GA. Thyroid hormone regulation of metabolism. *Physiol Rev* 94: 355–382, 2014.
90. Mustonen AM, Nieminen P, Puukka M, Asikainen J, Saarela S, Karonen SL, Kukkonen JVK, Hyvärinen H. Physiological adaptations of the raccoon dog (*Nyctereutes procyonoides*) to seasonal fasting-fat and nitrogen metabolism and influence of continuous melatonin treatment. *J Comp Physiol B* 174: 1–12, 2004.
91. Mustonen AM, Pyrkönen T, Puukka M, Asikainen J, Hänninen S, Mononen J, Nieminen P. Physiological adaptations to fasting in an actively wintering canid, the Arctic blue fox (*Alopex lagopus*). *J Exp Zool A Comp Exp Biol* 305: 32–46, 2006.
92. Nagayama Y. Animal models of graves' hyperthyroidism. *Endocr J* 52: 385–394, 2005.
93. Magnus TH, Henderson NE. Thyroid hormone resistance in hibernating ground squirrels, *Spermophilus richardsoni*. I. Increased binding of triiodo-L-thyronine and L-thyroxine by serum proteins. *Gen Comp Endocrinol* 69: 352–360, 1988.
94. Magnus TH, Henderson NE. Thyroid hormone resistance in hibernating ground squirrels, *Spermophilus richardsoni*. II. Reduction of hepatic nuclear receptors. *Gen Comp Endocrinol* 69: 361–371, 1988.
95. Nieminen P, Saarela S, Pyrkönen T, Asikainen J, Mononen J, Mustonen AM. Endocrine response to fasting in the overwintering captive raccoon dog (*Nyctereutes procyonoides*). *J Exp Zool A Comp Exp Biol* 301: 919–929, 2004.
96. Noren DP, Crocker DE, Williams TM, Costa DP. Energy reserve utilization in northern elephant seal (*Mirounga angustirostris*) pups during the postweaning fast: size does matter. *J Comp Physiol B* 173: 443–454, 2003.
97. O'Mara BA, Dittrich W, Lauterio TJ, Germain DLS. Pretranslational regulation of type I 5'-deiodinase by thyroid hormones and in fasted and diabetic rats. *Endocrinology* 133: 1715–1723, 1993.
98. Obregon MJ. Adipose tissues and thyroid hormones. *Front Physiol* 5: 479, 2014.
99. Ortega E, Koska J, Pannacchiulli N, Bunt JC, Krakoff J. Free triiodothyronine plasma concentrations are positively associated with insulin secretion in euthyroid individuals. *Eur J Endocrinol* 158: 217–221, 2008.
100. Ortiz CL, Costa D, Le Boeuf BJ. Water and energy flux in elephant seal pups fasting under natural conditions. *Physiol Zool* 51: 166–178, 1978.
101. Ortiz RM, Houser DS, Wade CE, Ortiz CL. Hormonal changes associated with the transition between nursing and natural fasting in northern elephant seals (*Mirounga angustirostris*). *Gen Comp Endocrinol* 130: 78–83, 2003.
102. Ortiz RM, MacKenzie DS, Worth GA. Thyroid hormone concentrations in captive and free-ranging West Indian manatees (*Trichechus manatus*). *J Exp Biol* 203: 3631–3637, 2000.
103. Owecki M, Nikisch E, Sowiński J. Hypothyroidism has no impact on insulin sensitivity assessed with HOMA-IR in totally thyroidectomized patients. *Acta Clin Belg* 61: 69–73, 2006.
104. Palumbo PJ, Wellik DL, Bagley NA, Nelson RA. Insulin and glucagon responses in the hibernating bear. *Int Conf Bear Res Manage* 5: 291–296, 1983.
105. Park J. The cell: a molecular approach, second edition. *Yale J Biol Med* 74: 361, 2001.
106. Pender C, Goldfine ID, Kulp JL, Tanner CJ, Mad-dux BA, MacDonald KG, Houmard JA, Youngren JF. Analysis of insulin-stimulated insulin receptor activation and glucose transport in cultured skeletal muscle cells from obese subjects. *Metab Clin Exp* 54: 598–603.
107. Prats-Puig A, Sitjar C, Ribot R, Calvo M, Clausell-Pomés N, Soler-Roca M, Soriano-Rodríguez P, Osiniri I, Ros-Miquel M, Bassols J, de Zegher F, Ibáñez L, López-Bermejo A. Relative hypoadiponectinemia, insulin resistance, and increased visceral fat in euthyroid prepubertal girls with low-normal serum free thyroxine. *Obesity (Silver Spring)* 20: 1455–1461, 2012.
108. Ren R, Jiang X, Zhang X, Guan Q, Yu C, Li Y, Gao L, Zhang H, Zhao J. Association between thyroid hormones and body fat in euthyroid subjects. *Clin Endocrinol* 80: 585–590, 2014.
109. Roos A, Bakker SJL, Links TP, Gans ROB, Woffenbuttel BHR. Thyroid function is associated with components of the metabolic syndrome in euthyroid subjects. *J Clin Endocrinol Metab* 92: 491–496, 2007.
110. Roti E, Minelli R, Salvi M. Thyroid hormone metabolism in obesity. *Int J Obes Relat Metab Disord* 24, Suppl 2: S113–S115, 2000.
111. Salvatore D, Simonides WS, Dentice M, Zavacki AM, Larsen PR. Thyroid hormones and skeletal muscle-new insights and potential implications. *Nat Rev Endocrinol* 4: 206–214, 2013.
112. Saunders J, Hall SEH, Sönksen PH. Glucose and free fatty acid turnover in thyrotoxicosis and hypothyroidism, before and after treatment. *Clin Endocrinol* 13: 33–44, 1980.
113. Seal US, Erickson AW, Mayo JG. Drug immobilisation of the carnivore. *Int Zoo Yearb* 10: 157–160, 1970.
114. Secor SM, Carey HV. Integrative physiology of fasting. *Compr Physiol* 6: 773–825, 2016.
115. Shen DC, Davidson MB, Kuo SW, Sheu WHH. Peripheral and hepatic insulin antagonism in hyperthyroidism. *J Clin Endocrinol Metab* 66: 565–569, 1988.
116. Singer PA, Nicoloff JT. Assessment of thyrotropin-releasing hormone and thyrotropin reserve in man. *J Clin Invest* 52: 1099–1107, 1973.
117. Sorisky A, Bell A, Gagnon A. TSH receptor in adipose cells. *Horm Metab Res* 32: 468–474, 2000.
118. Spencer CA, Lum SMC, Wilber JF, Kaptein EM, Nicoloff JT. Dynamics of serum thyrotropin and thyroid hormone changes in fasting. *J Clin Endocrinol Metab* 56: 883–888, 1983.
119. Spitzweg C, Joba W, Hunt N, Heufelder AE. Analysis of human thyrotropin receptor gene expression and immunoreactivity in human orbital tissue. *Eur J Endocrinol* 136: 599–607, 1997.

120. Stanická S, Vondra K, Pelikánová T, Vlcěk P, Hill M, Zamrazil V. Insulin sensitivity and counter-regulatory hormones in hypothyroidism and during thyroid hormone replacement therapy. *Clin Chem Lab Med* 7: 715–720, 2005.
121. Takeuchi Y, Suzuki D, Oda SI, Refetoff S, Seki K, Tsunekawa K, Kasahara T, Murakami M, Murata Y. Unique regulation of thyroid hormone metabolism during fasting in the house musk shrew (*Suncus murinus*, Insectivora: Soricidae). *Gen Comp Endocrinol* 146: 236–241, 2006.
122. Tomasi TE, Hellgren EC, Tucker TJ. Thyroid hormone concentrations in black bears (*Ursus americanus*): hibernation and pregnancy effects. *Gen Comp Endocrinol* 109: 192–199, 1998.
123. Tuzcu A, Bahceci M, Gokalp D, Tuzun Y, Gunes K. Subclinical hypothyroidism may be associated with elevated high-sensitive C-reactive protein (low grade inflammation) and fasting hyperinsulinemia. *Endocr J* 52: 89–94, 2005.
124. Singh UP, Krishna A, Bhatnagar KP. Seasonal changes in thyroid activity in the female sheath-tailed bat, *Taphozous longimanus* (Chiroptera: Emballonuridae). *Acta Biol Hung* 3: 267–278, 2002.
125. Valyasevi RW, Harteneck DA, Dutton CM, Bahn RS. Stimulation of adipogenesis, peroxisome proliferator-activated receptor- γ (PPAR γ), and thyrotropin receptor by PPAR γ agonist in human orbital preadipocyte fibroblasts. *J Clin Endocrinol Metab* 87: 2352–2358, 2002.
126. Vatner DF, Weismann D, Beddow SA, Kumashiro N, Erion DM, Liao XH, Grover GJ, Webb P, Phillips KJ, Weiss RE, Bogan JS, Baxter J, Shulman GI, Samuel VT. Thyroid hormone receptor- β agonists prevent hepatic steatosis in fat-fed rats but impair insulin sensitivity via discrete pathways. *Am J Physiol Endocrinol Metab* 305: E89–E100, 2013.
127. Vázquez-Medina JP, Crocker DE, Forman HJ, Ortiz RM. Prolonged fasting does not increase oxidative damage or inflammation in postweaned northern elephant seal pups. *J Exp Biol* 213: 2524–2530, 2010.
128. Verrier D, Atkinson S, Guinet C, Groscolas R, Arnould JPY. Hormonal responses to extreme fasting in subantarctic fur seal (*Arctocephalus tropicalis*) pups. *Am J Physiol Regul Integr Comp Physiol* 302: R929–R940, 2012.
129. Viscarra JA, Champagne CD, Crocker DE, Ortiz RM. 5'AMP-activated protein kinase activity is increased in adipose tissue of northern elephant seal pups during prolonged fasting-induced insulin resistance. *J Endocrinol* 209: 317–325, 2011.
130. Viscarra JA, Rodriguez R, Vazquez-Medina JP, Lee A, Tift MS, Tavoni SK, Crocker DE, Ortiz RM. Insulin and GLP-1 infusions demonstrate the onset of adipose-specific insulin resistance in a large fasting mammal: potential glucogenic role for GLP-1. *Physiol Rep* 1: e00023, 2013.
131. Viscarra JA, Rodriguez R, Vazquez-Medina JP, Lee A, Tift MS, Tavoni SK, Crocker DE, Ortiz RM. Insulin and GLP-1 infusions demonstrate the onset of adipose-specific insulin resistance in a large fasting mammal: potential glucogenic role for GLP-1. *Physiol Rep* 1: e00023, 2013.
132. Viscarra JA, Vázquez-Medina JP, Crocker DE, Ortiz RM. Glut4 is upregulated despite decreased insulin signaling during prolonged fasting in northern elephant seal pups. *Am J Physiol Regul Integr Comp Physiol* 300: R150–R154, 2011.
133. Viscarra JA, Vázquez-Medina JP, Rodriguez R, Champagne CD, Adams SH, Crocker DE, Ortiz RM. Decreased expression of adipose CD36 and FATP1 are associated with increased plasma non-esterified fatty acids during prolonged fasting in northern elephant seal pups (*Mirounga angustirostris*). *J Exp Biol* 215: 2455–2464, 2012.
134. Vyakaranam S, Vanaparthi S, Nori S, Palarapu S, Bhongir AV. Study of insulin resistance in subclinical hypothyroidism. *Intl J Hlth Sci Res* 4: 147–153, 2014.
135. Waring AC, Rodondi N, Harrison S, Kanaya AM, Simonsick EM, Miljkovic I, Satterfield S, Newman AB, Bauer DC, The Health, Aging, And Body Composition Study. Thyroid function and prevalent and incident metabolic syndrome in older adults: The Health, Aging, And Body Composition Study. *Clin Endocrinol* 76: 911–918, 2012.
136. Weinstein SP, O'Boyle E, Fisher M, Haber RS. Regulation of GLUT2 glucose transporter expression in liver by thyroid hormone: evidence for hormonal regulation of the hepatic glucose transport system. *Endocrinology* 135: 649–654, 1994.
137. Williams CT, Goropashnaya AV, Buck CL, Fedorov VB, Kohl F, Lee TN, Barnes BM. Hibernating above the permafrost: effects of ambient temperature and season on expression of metabolic genes in liver and brown adipose tissue of arctic ground squirrels. *J Exp Biol* 214: 1300–1306, 2011.
138. Wrutniak-Cabello C, Casas F, Cabello G. Thyroid hormone action in mitochondria. *J Mol Endocrinol* 26: 67–77, 2001.
139. Zaballos MA, Garcia B, Santisteban P. G β γ dimers released in response to thyrotropin activate phosphoinositide 3-kinase and regulate gene expression in thyroid cells. *Mol Endocrinol* 22: 1183–1199, 2008.