

Peripheral Regulation of Energy Metabolism by Thyroid Hormones

Jens Mittag

*Department of Cell & Molecular Biology, Karolinska Institutet, Stockholm, Sweden**Reviewing Editor: Graham Williams*

The author declares no conflict of interest.

Correspondence to:

Jens Mittag
Karolinska Institutet
Dept. Cell & Molecular Biology
von Eulers väg 3
17177 Stockholm - Sweden
Phone: +46(0)8-52487353
Email: jens.mittag@ki.se

ABSTRACT

Thyroid hormone is long known as an important regulator of metabolism. It exerts general effects such as increased cycling of metabolites and stimulation of ATP turnover in spite of reduced efficiency of oxidative phosphorylation, but also very specific effects in peripheral tissues. This article reviews the most relevant metabolic effects of thyroid hormone in peripheral tissues, including the specific contributions of the two different thyroid hormone receptor isoforms. Special focus is put on the thermogenic effects of thyroid hormone in muscle and brown adipose tissue as well as the exclusive role of the thyroid hormone receptor β in hepatic cholesterol metabolism.

Keywords: thermogenesis, brown adipose tissue, liver, glucose, metabolism, cholesterol

Introduction

It is known for over 100 years that thyroid hormone (TH) has a major impact on metabolism (1), and the metabolic rate has long been used as an indicator for the thyroid state. The connection between TH and metabolism becomes most evident in patients substituted with thyroxine (T4): after initiation of the treatment increased oxygen consumption is registered after 24 hours, reaching maximal levels after 4 days (2). Moreover, minimal dose changes, which do not move the serum free T4 out of the normal range, are readily detectable as changes in the metabolic rate (3). The higher energy expenditure caused by TH is usually accompanied by increased appetite; if food is restricted, weight loss occurs (4). This has led to the idea to use TH as diet pill, but the unwanted side effects such as tachycardia, muscle loss and osteoporosis outweigh the beneficial effects.

TH and its receptors

Two forms of TH are secreted from the thyroid gland: the prohormone T4 and the receptor-active form T3. Within the target tissue, T3 levels are fine-tuned by the activation or inactivation through deiodinating enzymes. While it has been assumed for long time that TH enters the cell due to its lipophilic properties, the view has changed with the recent discovery of specific TH transporters such as MCT8 or OATP14 (for review see (5)).

Within the cell, most effects of TH are mediated by nuclear TH receptors (TRs), which are encoded by the two distinct genes TR α and TR β . It is still a matter of controversy whether the TR α 1 and TR β isoforms can fully compensate for each other; however, the degree of redundancy seems to depend to a large extent on the level of TR isoform coexpression within a given celltype. The expression of TR α 1 is high in e.g. brown adipose tissue, skeletal muscle, brain and heart, while TR β is predominantly expressed in liver and kidney (for review see (6)).

A special feature of TRs is the ability to actively repress or activate target genes as aporeceptors in the absence of the ligand T3. This activity is the reason for the relatively mild phenotype of TR knockout mice (7-9) compared to mutants lacking TH or carrying mutations that impair TH binding to the TRs (10-14). The aporeceptor-function and the widespread partially overlapping expression

of the TRs make the interactions of TH with peripheral metabolism extremely complex including simultaneous actions at many different levels.

Metabolic Lessons from Animal Models

In most of the available animal models, targeting of the TR isoforms occurs in all tissues, thus the corresponding phenotypes are difficult to interpret. For instance, mice carrying a mutation in TR β which abolishes binding of T₃, or transgenic mice expressing a mutant human TR β both exhibit reduced body weight and size, but also high levels of TH due to the impaired negative feedback control of the pituitary (15, 16). Therefore, the metabolic effects are difficult to assign to a specific tissue and can be caused by either the high levels of TH acting on the intact TR α 1 or by the mutant TR β itself.

The situation is likewise complex for TR α 1. So far, four different mutations have been introduced into TR α 1, and the phenotypes range from metabolically lean (17), dwarfism with impaired adipogenesis (18, 19) or reduced fat content (20), to animals with visceral adiposity and increased fasting glucose and insulin (21, 22). As serum TH levels are surprisingly normal in these animals, the differences seem to be caused by the location of the mutation within the TR α 1 itself, which may affect interactions with cofactors or nuclear receptors such as PPARs (21).

However, hypothyroid mice as well as mice lacking all functional TRs show decreased metabolism (23). Furthermore, TR α 1^{-/-}-TR β ^{-/-} double mutants do no longer respond metabolically to TH. These observations indicate that the metabolic effects of TH are mediated largely by TR α 1 and TR β (23) and underline the importance of TH for the maintenance of normal metabolism.

Overall Effects of TH

TH generally enhances the turnover of lipids, carbohydrates and proteins, sometimes even simultaneously in reverse metabolic pathways (metabolic cycling). While fatty acids can be used up in this process, no protein is used for calorogenesis and the excretion of nitrogen as well as renal gluconeogenesis remain constant (2). Metabolic cycling, however, accounts for only 15% of

the increase in resting energy expenditure after TH administration (2), indicating that other processes such as ATP wasting and reduced efficiency of oxidative phosphorylation play a more important role in mediating the metabolic effects of the hormone.

The main energy waste is achieved by a combined increase of ATP consuming proteins, such as Na^+/K^+ - or Ca^{2+} -ATPases, and a stimulation of the ATP synthesizing machinery (3). TH enhances the mitochondrial oxidation capacity by e.g. increasing the amount of the adenine-nucleotide translocase 2 (ANT2), which transports ADP in and ATP out of the mitochondria (24), and the cytochromes c and c1 (25), which are part of the oxidizing machinery.

Simultaneously, the efficiency of the ATP synthesis itself is reduced; consequently, more fuel is needed for the same amount of biochemical work. In certain tissues such as the brown fat, this is achieved by a reduction of the coupling efficiency between mitochondrial proton gradient and ATP production through Uncoupling Protein 1 (UCP1). This protein generates artificial leaks in the mitochondrial membrane; however, UCP-independent mechanisms have also been reported (26). Moreover, at the cytosolic level, TH increases the expression of glycerol-3-phosphate dehydrogenase (24), which participates in one of the two shuttle systems that deliver NADH to the mitochondria. This shuttle system, which only yields 2 ATP molecules per NADH, is consequently preferred over the malate-aspartate shuttle, which generates 3 ATP per NADH (3). Thus, the efficiency of ATP production is additionally hampered.

Effects on the Adipose Tissue

In adipose tissue, TH stimulates lipolysis and lipogenesis simultaneously, which is in line with the concept of metabolite cycling. Lipolysis is enhanced by TH through a raised activity of hormone-sensitive lipase and an increased sensitivity of the adipose tissue to adrenergic stimulation, leading to higher levels of free-fatty acids in the serum. The expression of lipogenic enzymes such as malic enzyme, spot14 or fatty acid synthetase is also increased already after a few hours; the first de novo synthesis of fatty acids is detected about 10 hours later (2).

Interestingly, the $\text{TR}\beta$ -selective agonist GC1 does not induce fat loss to the same extent as T3, despite a similar increase in oxygen consumption (27). This demonstrates an important role for

TR β in the initial raise in energy expenditure, which differs from TR α 1-dependent adipose tissue activation. Indeed, the activity of the adipose tissue accounts for less than 5% of the increase in oxygen consumption after T3 administration, suggesting that the effects of TH on this tissue are a more long-term metabolic response. This correlates with the fact that the compensating food intake after TH treatment does not occur until 4 days later, most probably as a consequence of the reduced secretion of the satiety hormone leptin from the shrinking adipose tissue.

The effects of TH differ in the two types of adipose tissue. While the white adipose tissue is mainly a fat store and the induction of lipolysis generates fatty acids for the export, the brown adipose tissue (BAT) uses lipolysis, de novo synthesis and import of free fatty acids as fuel to maintain body temperature. TH does not induce thermogenesis by itself, but it is essential for the proper activation of this tissue (26). The activity of the BAT is mainly controlled by sympathetic signaling via the β 3-adrenergic receptor and completely dependent on UCP1; in its absence there is almost no sympathetic inducible thermogenesis (28). As there is good evidence that BAT also exists in humans (29), the thermogenic role of TH in mice and men will be elucidated in greater detail.

Thermogenic Effects of TH

TH stimulates obligatory thermogenesis (generated by basal metabolism) and is essential for facultative thermogenesis (generated by specialized mechanisms with the purpose to maintain body temperature) (3, 30). The latter is induced, if obligatory thermogenesis is not sufficient to maintain body temperature, and is divided into a fast response by e.g. muscle shivering and a slow but more long-lasting response through the BAT. The BAT response is based on heat production through uncoupling; mice lacking UCP1 loose more than 10°C body temperature when exposed to cold, whereas mice with an ablation of 60-70% of BAT are still cold resistant (31).

Interestingly, TR α 1^{-/-}-TR β ^{-/-} double knockout mice and hypothyroid mice such as the *hyt/hyt* mouse cannot survive cold at all (23, 32). While the BAT response is severely impaired in hypothyroid animals (33), UCP1 is inducible by adrenergic signaling in the TR α 1^{-/-}-TR β ^{-/-} double mutants (23). However, this is still not sufficient for survival in the cold; thus, proper TH signaling seems to be more important than BAT functionality alone.

Furthermore, TR aporeceptor activity plays an important role in this process: the T3 induced relief of apo-TR mediated repression seems required for sympathetic stimulation. While the UCP1 induction is restored by T3 and GC1 in hypothyroid mice, the sympathetic response is only rescued with T3. This indicates that TR β is involved in controlling UCP1 expression, while the adrenergic signaling is modulated mainly by TR α 1 signaling (33).

Vice versa, the adrenergic activation enhances TH signaling by inducing the T4 activating enzyme deiodinase type II (D2) (34), which in turn produces enough T3 to saturate all TRs in BAT (35). In the absence of D2, BAT adrenergic response is impaired. Consequently, D2 $^{-/-}$ mice develop hypothermia if exposed to cold, but still survive in contrast to hypothyroid animals, as the thermogenesis by shivering is not affected (36).

In summary, TRs exert well-defined tissue- and isoform specific roles in maintaining body temperature (Table 1). However, as mutations in TR α 1 also affect the sympathetic output from the brain (17), the central effects of TH on thermogenesis and metabolism might be underestimated to date. In addition, yet unknown TH effects on the vascular system might contribute to explain the reduced body temperature despite increased oxygen consumption in some animal models.

Animal Model	Body Temperature (relative to controls)	Comment
hyt/hyt	-2.5°C	severely hypothyroid, cold intolerant
TR β $^{-/-}$	normal	increased TH levels, not cold sensitive
TR α 1 $^{-/-}$	-0.5°C	not cold sensitive
TR α 0/0	-0.4°C	no facultative thermogenesis at room temperature, but higher O ₂ consumption
TR α 2 $^{-/-}$	+0.4°C	overexpress TR α 1
TR α 1R384C	-0.9°C	despite higher O ₂ consumption
TR α 1P398H	-0.5°C	obese
TR α 1L400R	normal	lean dwarfs
TR α 1 $^{-/-}$ -TR β $^{-/-}$	-0.4°C	increase metabolism and UCP1 upon cold, but still cold intolerant

Table 1: Body temperature of animal models with impairments in TH signaling (9, 17, 20, 22, 23, 32, 37-39)

Effects on the Muscle

The muscle is a versatile tissue regarding its use of metabolites. While the resting muscle consumes fatty acids, the active muscle requires large amounts of glucose. TH mainly affects muscle glucose metabolism; it increases glycolysis and almost doubles the amount of the insulin dependent glucose transporter GLUT4 on the cell surface (40). Consequently, the import, the flux through the Krebs-Cycle, and the oxygen consumption are all elevated; however, as the mitochondrial efficiency is also reduced by TH, the overall ATP production remains almost constant (41). Moreover, TH promotes further waste of energy by increasing the protein Ca^{2+} -ATPase, which transports Ca^{2+} from the cytosol into the sarcoplasmic reticulum (SR), and simultaneously raising the levels of the ryanodine receptor, which mediates the Ca^{2+} release from the SR back into the cytosol (42, 43). This mechanism alone accounts for an almost 2-fold increase in oxygen consumption between a hypo- and a hyperthyroid muscle (43), which raises the question for a physiological function as the muscle is dependent on an efficient ATP supply for proper function. However, as the muscle is also the first line of defence against hypothermia and TH actions in the muscle are absolutely required for survival in the cold, in the eye of TH the muscle is predominantly seen as a thermo- rather than a movement generator.

Effects on the Heart

It is well known that the consequences of thyrotoxicosis on the heart such as tachycardia closely resemble those of catecholamine excess; but surprisingly, catecholamine levels are normal if not lowered in hyperthyroidism. This suggests that TH increases cardiac responsiveness to catecholamines; however, not at the level of the adrenergic receptors (44).

The direct molecular effects of TH include an increase in HCN2 ion channels as well as an elevation of myosin heavy chain and SR- Ca^{2+} -ATPase 2, thus leading to an enhanced cardiac output and decreased the relaxation time (45). These effects are not observed with the $\text{TR}\beta$ selective compound GC1, underlining that $\text{TR}\alpha 1$ of major importance in the heart (27, 46). Correspondingly, mice lacking $\text{TR}\alpha 1$ have a decreased heart rate, while those overexpressing

TR α 1 exhibit an increased heart rate (9, 39). As for the BAT, any central effects of TH might additionally affect cardiac function e.g. via changes in the autonomic nervous system.

Effects on the Liver

As the liver acts at the crossroads of many metabolic pathways – the most important one being glucose homeostasis - it is not surprising that more than 5% of all genes expressed in the liver are regulated by TH (47, 48). These targets mediate general T3-effects such as increased oxygen consumption and ATP turnover (49), but also shift metabolic processes from glycogen synthesis to glycogenolysis and from glycolysis to gluconeogenesis, thus enhancing the endogenous hepatic glucose production (50). Moreover, T3 stimulates enzymes involved in lipogenesis such as malic enzyme, glucose-6-phosphate dehydrogenase and fatty acid synthetase. Although many of these target genes show redundant function for TR α 1 and TR β in the rodent liver (51), some such as spot14 (52) are predominantly regulated by TR β , the isoform accounting for 80% of hepatic T3 binding capacity (53).

One pathway exclusively regulated by TR β is cholesterol metabolism. Again, TH affects both ends: it induces the rate-limiting enzyme HMG-CoA reductase, thus stimulating de novo cholesterol synthesis, but also increases the expression of the LDL-receptor and CYP7A, the rate-limiting enzyme in bile-acid synthesis from cholesterol (54, 55). Together, this leads to a better clearance of serum LDL-cholesterol and an increased cholesterol breakdown.

Consequently, hypothyroidism is associated with hypercholesterolemia in men and mice due to a reduced clearance of serum LDL-cholesterol and a reduced bile-acid production by CYP7A (56). The TR β dependency of this process can be used to ameliorate this condition by the administration of the TR β selective compound GC1, which reduces serum cholesterol by 25% and leads to an increased faecal excretion of bile-acids (46, 57). Moreover, it reverses fatty livers and reduces the hepatic triglyceride content, indicating an important exclusive role of TR β also in fatty acid metabolism (58). Surprisingly, GC1 was found to be even more efficient than T3 in this context (46), which suggests opposite roles of TR α 1 and TR β in hepatic lipid metabolism. A similar reverse

effect of the TRs was observed in the regulation of PEPCK, the rate-limiting enzyme in gluconeogenesis (Vujovic, Vennström & Mittag, unpublished observations). These unique isoform-specific effects might be partially caused by the zoned hepatic expression of the TRs: TR β is limited to areas around the central vein, while TR α 1 is more widespread and the only periportal TR isoform (59-61). As a consequence, the isoforms cannot compensate for each other and might interact with different cellular cofactors in the different hepatic zones.

Despite the many well investigated direct effects of TH on hepatic gene expression, it should be kept in mind that TH also affects several hepatic metabolic pathways in an indirect manner, e.g. via the autonomic nervous system (62) or by enhancing the effects of other hormones such as insulin, glucagons or glucocorticoids (25).

Concluding Remarks

Besides the overall and long-known effects such as increase metabolic cycling and ATP turnover, many specific effects of TH in peripheral metabolism were identified over the last years, using e.g. novel TR β selective compounds and animal models with defective TH signaling (see Table 2).

Tissue	TR isoform	T3 Effect
Overall		1. metabolite cycling \uparrow 2. ATP use and production \uparrow 3. efficiency of ATP production \downarrow
White Fat	TR α 1+TR β	lipolysis, lipogenesis, export of FFA \uparrow
Brown Fat	TR α 1	adrenergic responsiveness, heat \uparrow
	TR β	uncoupling, lipolysis, heat \uparrow
Skeletal Muscle	TR α 1 \gg TR β	Ca ²⁺ cycling, glucose & ATP use \uparrow oxygen consumption, heat \uparrow
Heart	TR α 1 \gg TR β	tachycardia, cardiac output \uparrow
Liver	TR β (80%) ¹	lipogenesis (malic enzyme, spot14, FAS) \uparrow gluconeogenesis (PEPCK) \uparrow glycogenolysis \uparrow glycolysis (pyruvate kinase) \downarrow glycogensynthesis \downarrow cholesterol \downarrow
	TR α 1 ²	gluconeogenesis \downarrow ?
Other	TR α 1	required for proper development and function of the autonomic nervous system
	TR α 1, TR β	effects of glucocorticoids, insulin, glucagons, catecholamines \uparrow

Table 2: Role of TH and TRs in different tissues; ¹perivenously ²periportally; FAS = fatty acid synthetase; FFA = free fatty acids; PEPCK = phosphoenol pyruvate carboxykinase

This identification of TR isoform specific functions opened the possibility to target defined metabolic pathways. The TR β selective compounds KB2115 and GC1, for instance, were shown to be very efficient in reducing serum cholesterol in different animal models without the characteristic cardiac effects of T3 (63, 64). Unfortunately, the Holy Grail, a TH based diet pill, is still not achieved, as weight loss is usually accompanied by a compensatory increased food intake and weight regain on drug withdrawal. However, recent discoveries of novel TH dependent pathways, such as the actions of bile acids on TH-dependent activation of the BAT (65) or the hypothermic effects of the TH-derivate 3-iodothyronamine (66), impressively demonstrate the outstanding role of TH in the regulation of metabolism and reminds us, in spite of the many novel metabolic regulators, not to forget the “old” players.

Acknowledgements

I thank Prof Björn Vennström, Milica Vujovic and Susi Dudazy for critically reading the manuscript. Furthermore, I am grateful for financial support from the Deutsche Forschungsgemeinschaft.

References

1. Magnus-Levy A. Uber den respiratorischen Gaswechsel unter dem Einfluss der Thyroidea sowie unter verschiedenen pathologischen Zuständen Berlin Klin Wochenschr 34:650-652, 1895
2. Oppenheimer JH, Schwartz HL, Lane JT, Thompson MP. Functional relationship of thyroid hormone-induced lipogenesis, lipolysis, and thermogenesis in the rat The Journal of clinical investigation 87:125-132, 1991
3. Silva JE. Thermogenic mechanisms and their hormonal regulation Physiological reviews 86:435-464, 2006
4. Mooradian AD. Age-related differences in body weight loss in response to altered thyroidal status Experimental gerontology 25:29-35, 1990
5. Friesema EC, Jansen J, Milici C, Visser TJ. Thyroid hormone transporters Vitamins and hormones 70:137-167, 2005

6. Yen PM. Physiological and molecular basis of thyroid hormone action *Physiological reviews* 81:1097-1142, 2001
7. Forrest D, Erway LC, Ng L, Altschuler R, Curran T. Thyroid hormone receptor beta is essential for development of auditory function *Nature genetics* 13:354-357, 1996
8. Gothe S, Wang Z, Ng L, Kindblom JM, Barros AC, Ohlsson C, Vennstrom B, Forrest D. Mice devoid of all known thyroid hormone receptors are viable but exhibit disorders of the pituitary-thyroid axis, growth, and bone maturation *Genes & development* 13:1329-1341, 1999
9. Wikstrom L, Johansson C, Salto C, Barlow C, Campos Barros A, Baas F, Forrest D, Thoren P, Vennstrom B. Abnormal heart rate and body temperature in mice lacking thyroid hormone receptor alpha 1 *The EMBO journal* 17:455-461, 1998
10. Flamant F, Poguuet AL, Plateroti M, Chassande O, Gauthier K, Streichenberger N, Mansouri A, Samarut J. Congenital hypothyroid Pax8(-/-) mutant mice can be rescued by inactivating the TRalpha gene *Molecular endocrinology (Baltimore, Md)* 16:24-32, 2002
11. Itoh Y, Esaki T, Kaneshige M, Suzuki H, Cook M, Sokoloff L, Cheng SY, Nunez J. Brain glucose utilization in mice with a targeted mutation in the thyroid hormone alpha or beta receptor gene *Proceedings of the National Academy of Sciences of the United States of America* 98:9913-9918, 2001
12. Mansouri A, Chowdhury K, Gruss P. Follicular cells of the thyroid gland require Pax8 gene function *Nature genetics* 19:87-90, 1998
13. Mittag J, Friedrichsen S, Heuer H, Polsfuss S, Visser TJ, Bauer K. Athyroid Pax8-/- mice cannot be rescued by the inactivation of thyroid hormone receptor alpha1 *Endocrinology* 146:3179-3184, 2005
14. O'Shea PJ, Williams GR. Insight into the physiological actions of thyroid hormone receptors from genetically modified mice *The Journal of endocrinology* 175:553-570, 2002
15. Kaneshige M, Kaneshige K, Zhu X, Dace A, Garrett L, Carter TA, Kazlauskaitė R, Pankratz DG, Wynshaw-Boris A, Refetoff S, et al. Mice with a targeted mutation in the thyroid hormone beta receptor gene exhibit impaired growth and resistance to thyroid hormone

- Proceedings of the National Academy of Sciences of the United States of America 97:13209-13214, 2000
16. Wong R, Vasilyev VV, Ting YT, Kutler DI, Willingham MC, Weintraub BD, Cheng S. Transgenic mice bearing a human mutant thyroid hormone beta 1 receptor manifest thyroid function anomalies, weight reduction, and hyperactivity *Molecular medicine* (Cambridge, Mass 3:303-314, 1997
 17. Sjogren M, Alkemade A, Mittag J, Nordstrom K, Katz A, Rozell B, Westerblad H, Arner A, Vennstrom B. Hypermetabolism in mice caused by the central action of an unliganded thyroid hormone receptor alpha1 *The EMBO journal* 26:4535-4545, 2007
 18. Kaneshige M, Suzuki H, Kaneshige K, Cheng J, Wimbrow H, Barlow C, Willingham MC, Cheng S. A targeted dominant negative mutation of the thyroid hormone alpha 1 receptor causes increased mortality, infertility, and dwarfism in mice *Proceedings of the National Academy of Sciences of the United States of America* 98:15095-15100, 2001
 19. Ying H, Araki O, Furuya F, Kato Y, Cheng SY. Impaired adipogenesis caused by a mutated thyroid hormone alpha1 receptor *Molecular and cellular biology* 27:2359-2371, 2007
 20. Quignodon L, Vincent S, Winter H, Samarut J, Flamant F. A point mutation in the activation function 2 domain of thyroid hormone receptor alpha1 expressed after CRE-mediated recombination partially recapitulates hypothyroidism *Molecular endocrinology* (Baltimore, Md 21:2350-2360, 2007
 21. Liu YY, Heymann RS, Moatamed F, Schultz JJ, Sobel D, Brent GA. A mutant thyroid hormone receptor alpha antagonizes peroxisome proliferator-activated receptor alpha signaling in vivo and impairs fatty acid oxidation *Endocrinology* 148:1206-1217, 2007
 22. Liu YY, Schultz JJ, Brent GA. A thyroid hormone receptor alpha gene mutation (P398H) is associated with visceral adiposity and impaired catecholamine-stimulated lipolysis in mice *The Journal of biological chemistry* 278:38913-38920, 2003
 23. Golozoubova V, Gullberg H, Matthias A, Cannon B, Vennstrom B, Nedergaard J. Depressed thermogenesis but competent brown adipose tissue recruitment in mice devoid

- of all hormone-binding thyroid hormone receptors *Molecular endocrinology* (Baltimore, Md 18:384-401, 2004
24. Dummler K, Muller S, Seitz HJ. Regulation of adenine nucleotide translocase and glycerol 3-phosphate dehydrogenase expression by thyroid hormones in different rat tissues *The Biochemical journal* 317 (Pt 3):913-918, 1996
 25. Weitzel JM, Iwen KA, Seitz HJ. Regulation of mitochondrial biogenesis by thyroid hormone *Experimental physiology* 88:121-128, 2003
 26. Cannon B, Nedergaard J. Brown adipose tissue: function and physiological significance *Physiological reviews* 84:277-359, 2004
 27. Villicev CM, Freitas FR, Aoki MS, Taffarel C, Scanlan TS, Moriscot AS, Ribeiro MO, Bianco AC, Gouveia CH. Thyroid hormone receptor beta-specific agonist GC-1 increases energy expenditure and prevents fat-mass accumulation in rats *The Journal of endocrinology* 193:21-29, 2007
 28. Matthias A, Ohlson KB, Fredriksson JM, Jacobsson A, Nedergaard J, Cannon B. Thermogenic responses in brown fat cells are fully UCP1-dependent. UCP2 or UCP3 do not substitute for UCP1 in adrenergically or fatty acid-induced thermogenesis *The Journal of biological chemistry* 275:25073-25081, 2000
 29. Nedergaard J, Bengtsson T, Cannon B. Unexpected evidence for active brown adipose tissue in adult humans *American journal of physiology* 293:E444-452, 2007
 30. Kim B. Thyroid hormone as a determinant of energy expenditure and the basal metabolic rate *Thyroid* 18:141-144, 2008
 31. Enerback S, Jacobsson A, Simpson EM, Guerra C, Yamashita H, Harper ME, Kozak LP. Mice lacking mitochondrial uncoupling protein are cold-sensitive but not obese *Nature* 387:90-94, 1997
 32. Endo T, Kobayashi T. Thyroid-stimulating hormone receptor in brown adipose tissue is involved in the regulation of thermogenesis *American journal of physiology* 295:E514-518, 2008

33. Ribeiro MO, Carvalho SD, Schultz JJ, Chiellini G, Scanlan TS, Bianco AC, Brent GA. Thyroid hormone--sympathetic interaction and adaptive thermogenesis are thyroid hormone receptor isoform--specific *The Journal of clinical investigation* 108:97-105, 2001
34. Silva JE, Larsen PR. Adrenergic activation of triiodothyronine production in brown adipose tissue *Nature* 305:712-713, 1983
35. Bianco AC, Maia AL, da Silva WS, Christoffolete MA. Adaptive activation of thyroid hormone and energy expenditure *Bioscience reports* 25:191-208, 2005
36. de Jesus LA, Carvalho SD, Ribeiro MO, Schneider M, Kim SW, Harney JW, Larsen PR, Bianco AC. The type 2 iodothyronine deiodinase is essential for adaptive thermogenesis in brown adipose tissue *The Journal of clinical investigation* 108:1379-1385, 2001
37. Gauthier K, Plateroti M, Harvey CB, Williams GR, Weiss RE, Refetoff S, Willott JF, Sundin V, Roux JP, Malaval L, et al. Genetic analysis reveals different functions for the products of the thyroid hormone receptor alpha locus *Molecular and cellular biology* 21:4748-4760, 2001
38. Johansson C, Gothe S, Forrest D, Vennstrom B, Thoren P. Cardiovascular phenotype and temperature control in mice lacking thyroid hormone receptor-beta or both alpha1 and beta *The American journal of physiology* 276:H2006-2012, 1999
39. Salto C, Kindblom JM, Johansson C, Wang Z, Gullberg H, Nordstrom K, Mansen A, Ohlsson C, Thoren P, Forrest D, et al. Ablation of TRalpha2 and a concomitant overexpression of alpha1 yields a mixed hypo- and hyperthyroid phenotype in mice *Molecular endocrinology* (Baltimore, Md 15:2115-2128, 2001
40. Leijendekker WJ, van Hardeveld C, Elzinga G. Heat production during contraction in skeletal muscle of hypothyroid mice *The American journal of physiology* 253:E214-220, 1987
41. Short KR, Nygren J, Barazzoni R, Levine J, Nair KS. T(3) increases mitochondrial ATP production in oxidative muscle despite increased expression of UCP2 and -3 *American journal of physiology* 280:E761-769, 2001

42. Connelly TJ, el-Hayek R, Sukhareva M, Coronado R. L-thyroxine activates the intracellular Ca²⁺ release channel of skeletal muscle sarcoplasmic reticulum *Biochemistry and molecular biology international* 32:441-448, 1994
43. Simonides WS, Thelen MH, van der Linden CG, Muller A, van Hardeveld C. Mechanism of thyroid-hormone regulated expression of the SERCA genes in skeletal muscle: implications for thermogenesis *Bioscience reports* 21:139-154, 2001
44. Kim B, Carvalho-Bianco SD, Larsen PR. Thyroid hormone and adrenergic signaling in the heart *Arquivos brasileiros de endocrinologia e metabologia* 48:171-175, 2004
45. Klein I, Ojamaa K. Thyroid hormone and the cardiovascular system *The New England journal of medicine* 344:501-509, 2001
46. Trost SU, Swanson E, Gloss B, Wang-Iverson DB, Zhang H, Volodarsky T, Grover GJ, Baxter JD, Chiellini G, Scanlan TS, et al. The thyroid hormone receptor-beta-selective agonist GC-1 differentially affects plasma lipids and cardiac activity *Endocrinology* 141:3057-3064, 2000
47. Feng X, Jiang Y, Meltzer P, Yen PM. Thyroid hormone regulation of hepatic genes in vivo detected by complementary DNA microarray *Molecular endocrinology (Baltimore, Md)* 14:947-955, 2000
48. Flores-Morales A, Gullberg H, Fernandez L, Stahlberg N, Lee NH, Vennstrom B, Norstedt G. Patterns of liver gene expression governed by TRbeta *Molecular endocrinology (Baltimore, Md)* 16:1257-1268, 2002
49. Harper ME, Brand MD. The quantitative contributions of mitochondrial proton leak and ATP turnover reactions to the changed respiration rates of hepatocytes from rats of different thyroid status *The Journal of biological chemistry* 268:14850-14860, 1993
50. Muller MJ, Seitz HJ. Rapid and direct stimulation of hepatic gluconeogenesis by L-triiodothyronine (T₃) in the isolated-perfused rat liver *Life sciences* 27:827-835, 1980
51. Yen PM, Feng X, Flamant F, Chen Y, Walker RL, Weiss RE, Chassande O, Samarut J, Refetoff S, Meltzer PS. Effects of ligand and thyroid hormone receptor isoforms on hepatic

- gene expression profiles of thyroid hormone receptor knockout mice *EMBO reports* 4:581-587, 2003
52. Kinlaw WB, Tron P, Witters LA. Thyroid hormone and dietary carbohydrate induce different hepatic zonation of both "spot 14" and acetyl-coenzyme-A carboxylase: a novel mechanism of coregulation *Endocrinology* 133:645-650, 1993
53. Weiss RE, Murata Y, Cua K, Hayashi Y, Seo H, Refetoff S. Thyroid hormone action on liver, heart, and energy expenditure in thyroid hormone receptor beta-deficient mice *Endocrinology* 139:4945-4952, 1998
54. Gullberg H, Rudling M, Forrest D, Angelin B, Vennstrom B. Thyroid hormone receptor beta-deficient mice show complete loss of the normal cholesterol 7alpha-hydroxylase (CYP7A) response to thyroid hormone but display enhanced resistance to dietary cholesterol *Molecular endocrinology (Baltimore, Md)* 14:1739-1749, 2000
55. Gullberg H, Rudling M, Salto C, Forrest D, Angelin B, Vennstrom B. Requirement for thyroid hormone receptor beta in T3 regulation of cholesterol metabolism in mice *Molecular endocrinology (Baltimore, Md)* 16:1767-1777, 2002
56. Beamer WJ, Eicher EM, Maltais LJ, Southard JL. Inherited primary hypothyroidism in mice *Science (New York, NY)* 212:61-63, 1981
57. Johansson L, Rudling M, Scanlan TS, Lundasen T, Webb P, Baxter J, Angelin B, Parini P. Selective thyroid receptor modulation by GC-1 reduces serum lipids and stimulates steps of reverse cholesterol transport in euthyroid mice *Proceedings of the National Academy of Sciences of the United States of America* 102:10297-10302, 2005
58. Perra A, Simbula G, Simbula M, Pibiri M, Kowalik MA, Sulas P, Cocco MT, Ledda-Columbano GM, Columbano A. Thyroid hormone (T3) and TRbeta agonist GC-1 inhibit/reverse nonalcoholic fatty liver in rats *Faseb J* 22:2981-2989, 2008
59. Zandieh Doulabi B, Platvoet-ter Schiphorst M, van Beeren HC, Labruyere WT, Lamers WH, Fliers E, Bakker O, Wiersinga WM. TR(beta)1 protein is preferentially expressed in the pericentral zone of rat liver and exhibits marked diurnal variation *Endocrinology* 143:979-984, 2002

60. Zandieh-Doulabi B, Dop E, Schneiders M, Schiphorst MP, Mansen A, Vennstrom B, Dijkstra CD, Bakker O, Wiersinga WM. Zonal expression of the thyroid hormone receptor alpha isoforms in rodent liver *The Journal of endocrinology* 179:379-385, 2003
61. Zandieh-Doulabi B, Platvoet-ter Schiphorst M, Kalsbeek A, Wiersinga WM, Bakker O. Hyper and hypothyroidism change the expression and diurnal variation of thyroid hormone receptor isoforms in rat liver without major changes in their zonal distribution *Molecular and cellular endocrinology* 219:69-75, 2004
62. Klieverik LP, Sauerwein HP, Ackermans MT, Boelen A, Kalsbeek A, Fliers E. Effects of thyrotoxicosis and selective hepatic autonomic denervation on hepatic glucose metabolism in rats *American journal of physiology* 294:E513-520, 2008
63. Baxter JD, Webb P, Grover G, Scanlan TS. Selective activation of thyroid hormone signaling pathways by GC-1: a new approach to controlling cholesterol and body weight *Trends in endocrinology and metabolism: TEM* 15:154-157, 2004
64. Berkenstam A, Kristensen J, Mellstrom K, Carlsson B, Malm J, Rehnmark S, Garg N, Andersson CM, Rudling M, Sjoberg F, et al. The thyroid hormone mimetic compound KB2115 lowers plasma LDL cholesterol and stimulates bile acid synthesis without cardiac effects in humans *Proceedings of the National Academy of Sciences of the United States of America* 105:663-667, 2008
65. Watanabe M, Houten SM, Mataka C, Christoffolete MA, Kim BW, Sato H, Messaddeq N, Harney JW, Ezaki O, Kodama T, et al. Bile acids induce energy expenditure by promoting intracellular thyroid hormone activation *Nature* 439:484-489, 2006
66. Scanlan TS, Suchland KL, Hart ME, Chiellini G, Huang Y, Kruzich PJ, Frascarelli S, Crossley DA, Bunzow JR, Ronca-Testoni S, et al. 3-Iodothyronamine is an endogenous and rapid-acting derivative of thyroid hormone *Nature medicine* 10:638-642, 2004