Stimulation of S14 mRNA and lipogenesis in brown fat by hypothyroidism, cold exposure, and cafeteria feeding: Evidence supporting a general role for S14 in lipogenesis and lipogenesis in the maintenance of thermogenesis

(thyroid hormone/fatty acid metabolism/rat)

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ABSTRACT   In liver, thyroid hormone rapidly induces S14 mRNA, which encodes a small acidic protein. This sequence is abundantly expressed only in lipogenic tissues and is thought to have some function in fat metabolism. In the euthyroid rat, we measured 20-fold higher levels of S14 mRNA in interscapular brown adipose tissue than liver. Furthermore, whereas in liver or epididymal fat, hypothyroidism resulted in an 80% fall in S14 mRNA, in brown fat the level of this sequence increased a further 3-fold. In all three tissues, the expression of S14 mRNA correlated well with lipogenesis, as assessed by ^3H_2O incorporation. Physiological activation of brown fat by chronic cold exposure or cafeteria feeding increased the concentration of S14 mRNA in this tissue and again this was accompanied by a greater rate of fatty acid synthesis. Overall, in liver and white and brown adipose tissue, S14 mRNA and lipogenesis were well correlated and strongly suggest a function of the S14 protein related to fat synthesis. These studies suggest that the S14 protein and lipogenesis may be important for thyroid hormone-induced and brown adipose tissue thermogenesis and that stimulation of these functions in hypothyroid brown fat is a consequence of decreased thyroid hormone-induced thermogenesis elsewhere.

In our efforts to elucidate the mechanism of action of thyroid hormone, our laboratory has recently focused attention on S14 mRNA. This product was initially identified by in vitro translation of hepatic mRNA (1) and was selected because of its extremely rapid response to 3,5,3'-triiodothyronine (T3) (2). Its cDNA has now been cloned (3) and used to study intensively the regulation of this sequence by thyroid hormone and diet (4, 5). Elevations in S14 mRNA are seen as early as 20 min after administration of T3 to hypothyroid animals (4), and increases in its nuclear precursor have been detected even earlier (6). The steady-state levels of S14 mRNAs are ≈20-fold higher in the hyper- as compared to hypothyroid rat.

At this time the identity of the S14 protein remains unknown, though available evidence is suggestive of a role in some aspect of fat metabolism. It is expressed at high levels only in liver and various fat depots, including lactating mammary gland (5). Its response to a variety of stimuli, including T3, a high carbohydrate diet, starvation, and diabetes, parallels that of lipogenic enzymes (7). However, searches of polynucleotide and protein data banks have revealed no significant homologies with the coding areas of the S14 gene, or its predicted peptide sequence, and therefore give no information concerning its identity.

In our studies of the tissue distribution of S14 mRNA, we were intrigued to find high levels of expression in brown adipose tissue (BAT) (5). BAT is a tissue that has the unique capacity to uncouple oxidative phosphorylation from ATP synthesis and thus consume energy stores simply to produce heat (8). It constitutes the principal site for nonshivering thermogenesis (9) and is also the site for diet-induced thermogenesis following overfeeding (10) in a number of small mammals. Although the thermogenic effects of thyroid hormones are well known (11), they do not uncouple respiration in BAT (12, 13) or, indeed, in any other tissue (11). However, thyroid hormone appears to be essential for the realization of the full thermogenic capacity of BAT. Thus, hypothyroid animals do not survive in the cold (14) and seem unable to increase respiration in BAT when stimulated by cold exposure or norepinephrine (15).

We have chosen to examine the regulation of expression of the S14 gene in BAT by thyroid hormones and by physiological activation of this tissue. These findings in BAT have been compared with parallel measurements in epididymal fat and liver and demonstrate a notable discordancy in regulation between tissues. However, the levels of S14 mRNA remain well correlated with the lipogenic activity of the respective tissues, strongly suggesting a lipogenic function for the S14 protein. Our results, when taken with previous investigations of BAT, indicate an increased basal activity of this tissue in the hypothyroid state. Furthermore, the enhanced synthesis of fatty acids appears to be an important component for the maintenance of thyroid hormone-induced and BAT thermogenesis.

METHODS

Animals. Male Sprague–Dawley (Biolabs, St. Paul, MN) rats weighing 175–300 g were used in all experiments and routinely housed in groups of four at 22–24°C, on a 12:12 light:dark cycle. Hypothyroidism was induced with 0.025% methimazole in the drinking water for 3 weeks, by which time no further weight gain was apparent and serum T3 was undetectable. Euthyroid animals were made hyperthyroid by injecting 15 μg of T3 per 100 g of body weight per day i.p. for 7 days. Norepinephrine was given as a single i.p. injection (40 μg/100 g of body weight), 4 hr before killing. Euthyroid animals were cold stressed at 5°C, in individual cages, either acutely for 4–24 hr or chronically for 3 weeks. Other rats were induced to overeat by presenting a cafeteria diet as described by others (16, 17). Four palatable foods were selected each day from a range of 20 (meat sandwiches, hot dogs, bacon,

Abbreviations: BAT, brown adipose tissue; T3, 3,5,3'-triiodothyronine.
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cheese, crackers, potato chips, peanut butter sandwiches, candy bars, sweetened breakfast cereals, cookies, marshmallows). Animals were kept on the diet for 2 weeks and had continued access to chow. Food intake was measured as the difference between that supplied and that spilt, and its caloric content was determined from food tables. The diet as consumed contained 42% carbohydrate, 44% fat, and 14% protein, as a percentage of total calories.

S14 mRNA Quantitation. Between 9:00 and 10:00, animals were anesthetized lightly with ether and blood was collected from the aorta. Liver, epididymal fat, interscapular BAT, and occasionally retroperitoneal fat were removed. The BAT was carefully dissected away from the adjoining white fat and muscle. Tissues were weighed, frozen in liquid nitrogen, and stored at $-80°C$. Total RNA was extracted in 4 M guanidinium thiocyanate and purified using cesium chloride centrifugation as described by Chirgwin *et al.* (18). RNA was further purified with organic extraction and washing with 3 M sodium acetate as described (4).

The coidentity of S14 mRNA in BAT and epididymal fat with the well-characterized hepatic sequences has been confirmed by RNA transfer gel analysis (5). In these experiments the level of S14 mRNA was quantitated by dot blot hybridization (4) using a specific S14 cDNA probe, excised with *Pst* I from pS14c2, which recognizes most of the translated portion of the mRNA (3). In the case of BAT, because of the high intensity of the signal, only 0.5–2.0 μg of total RNA were dotted.

Lipogenesis. Animals were injected between 9:00 and 10:00 with 4 mCi (1 Ci = 37 GBq) of ^3H_2O diluted to 200 μl with saline (initial specific activity, 100 mCi/ml; Amersham). They were killed 30 min later, a time at which ^3H incorporation into tissue fatty acids was still linear and plasma levels of labeled fatty acids were negligible. Tissues were removed as described above and ^3H incorporation into fatty acids was determined by the method of Stansbie *et al.* (19). The specific activity of plasma water was determined and the rate of fatty acid synthesis was calculated as μmol of H incorporated per g/hr.

Other Assays. BAT nuclear T3 receptors were quantitated by using modifications of an *in vivo* technique (20). Briefly, animals were injected i.v. with 25 ng of [^{125}I]T3 (specific activity, 568 $\mu Ci/\mu g$; Abbott) and increasing amounts (0.2–200 μg) of nonradioactive T3. They were killed 1 hr later, at the equilibrium time point and bled. The BAT was removed and dissected clear of other tissue. The tissue was homogenized in 0.25 M sucrose/20 mM Tris·HCl, pH 7.8/1 mM $MgCl_2$ and centrifuged at 8000 × g for 15 min. The supernatant was discarded and the pellet was resuspended in the same buffer and centrifuged at 800 × g for 10 min. The nuclear pellet was then purified by washing twice in the sucrose buffer with 1% Triton X-100, and the radioactivity in the final pellet was determined. Nonspecific binding was assessed by using animals that were also injected with 200 μg/100 g of body weight nonradioactive T3.

Plasma T3 concentrations in selected animals were assayed by the method of Surks *et al.* (21). Statistical differences between groups were determined by analysis of variance, using the Student–Newman–Keuls procedure.

RESULTS

S14 mRNA in Adipose Tissue and Its Regulation by Thyroid Hormone. Euthyroid adipose tissues contain high concentrations of S14 mRNA (Table 1). Epididymal and retroperitoneal fat had about the same amount, which was 10-fold more than that seen in liver. In BAT, the concentration was 2-fold higher still, which meant that this tissue has the highest expression of S14 mRNA of any yet examined in the rat.

We next investigated the response of S14 mRNA in adipose tissue to chronic variations in thyroid state. The changes seen in epididymal and retroperitoneal fat were similar to those found in liver (Table 1). In methimazole-treated hypothyroid rats, levels of S14 mRNA in these tissues were about one-fifth of those seen in euthyroid animals. Induction of hyperthyroidism resulted in a further, $\approx$2-fold stimulation. However, in BAT, the already high expression of S14 mRNA in the euthyroid state was elevated a further 3-fold when the animals were made hypothyroid ($P < 0.001$). A small but insignificant increase was also observed in the euthyroid to hyperthyroid transition (Table 1). This discontinuous response of BAT S14 mRNA to increasing thyroid hormone levels awaits explanation and suggests a role for other regulators of this sequence in BAT.

This opposite response of S14 mRNA to hypothyroidism in BAT prompted us to examine the effects of short-term treatment of hypothyroid animals with a receptor-saturating dose of T3 (Table 2). The well-described (2, 4) rapid response of the hepatic sequence was reproduced. The response in epididymal fat was slower, a significant increase only being observed at the 24-hr time point. However, no significant changes were seen in the levels of S14 mRNA in BAT, where throughout the experiment they were maintained at $\approx$2-fold the euthyroid levels.

Measurement of T3 Receptors in BAT. Since there were no previous reports of T3 receptors in BAT, it seemed possible that the lack of short-term regulation of S14 mRNA by T3 could be explained by an absence of receptors, with the rise seen with chronic hypothyroidism being a secondary phenomenon. However, accumulation of radiolabeled T3 was detectable in Triton X-100-washed nuclei prepared from BAT, and this radioactivity was greatly diminished in animals that were simultaneously injected with a large dose of nonradioactive T3. Scatchard analysis of the binding data indicated an association constant of 1.5 × 10^{11}, with a receptor concentration of 0.74 pmol/mg of DNA, which is comparable to the amount seen in liver (20). Thus, the lack of a short-term effect of T3 in BAT could not be explained by a lack of receptors.

Effect of Thyroidal State on Lipogenesis. Previous studies have suggested a link between S14 mRNA expression and

Table 1. Effect of thyroid state on expression of S14 mRNA in lipogenic tissues of the rat

Tissue	Euthyroid	Hypothyroid	Hyperthyroid
Liver	12.6 ± 1.6 (14)	3.56 ± 0.68* (9)	30.0 ± 3.4* (4)
Epididymal fat	127.4 ± 14.2 (24)	28.4 ± 5.2† (15)	240.7 ± 52.0* (5)
Retroperitoneal fat	130.0 ± 11.2 (3)	21.6 ± 3.5† (5)	302.7 ± 70.3‡ (3)
BAT	226.6 ± 15.7 (23)	606.2 ± 108.7† (15)	286.5 ± 16.7 (4)

Data are expressed as mean ± SEM per μg of RNA, as percentages of an internal standard included on all blots. Values in parentheses indicate the number of separate estimations, all from different animals.
*$P < 0.005$ versus corresponding euthyroid tissue.
†$P < 0.001$ versus corresponding euthyroid tissue.
‡$P < 0.05$ versus corresponding euthyroid tissue.

Table 2. Effects of T3 injection on expression of S14 mRNA

Tissue	Hypothyroid	4-hr T3	24-hr T3	24-hr saline
Liver	2.5	33.2	51.4	ND
Epididymal fat	28.0 ± 7.8	36.9 ± 13.4	115.2 ± 5.7*	27.2 ± 3.4
BAT	472.0 ± 60.6	381.0 ± 42.7	614.0 ± 83.1	427.0 ± 74.4

Hypothyroid rats were injected with 200 μg/100 g of body weight T3. Values are expressed per μg of RNA, as percentages of an internal standard included on all blots. For liver, the mean of two estimations is given; for adipose tissue, data are expressed as mean ± SEM, $n = 4$. ND, not done.
*$P < 0.001$ versus saline-injected.

fatty acid synthesis (5, 7). If this were maintained in BAT, then this tissue would be more lipogenically active in hypothyroid animals than euthyroid, in contradistinction to liver and white fat. To test this possibility, hypo- or euthyroid animals were injected with ^3H_2O and half an hour later the relevant tissues were removed and ^3H incorporation into fatty acids was measured. In liver and epididymal fat, hypothyroidism resulted in a fall in fatty acid synthesis (Table 3), consistent with the lower levels of S14 mRNA. However, in BAT, the lipogenic activity of the hypothyroid tissue was double that in the euthyroid, so that in this organ, also, the parallelism with S14 mRNA was maintained.

Effects of Cold Exposure. We next examined the consequences of physiological activation of BAT on S14 expression. In the short term, levels of S14 mRNA in euthyroid rats were unaffected by exposure to 5°C (4 hr, 82% ± 25%; 24 hr, 91% ± 29%, both as % of controls) or injection of norepinephrine (4 hr, 82% ± 22%). The period of cold exposure was then extended to 3 weeks. The animals grew at the same rate as their room temperature controls, while consuming twice as much food (data not shown). The mass of their interscapular brown fat was increased by a factor of 4 (Table 4). In liver, neither the concentration of S14 mRNA nor fatty acid synthesis per g of tissue changed significantly with cold exposure. However, both parameters were increased in the two fat depots examined (Table 4). Epididymal fat S14 mRNA and lipogenesis both rose 2- to 3-fold. In BAT, S14 mRNA went up 3-fold and lipogenesis was 9-fold higher than in the room temperature controls.

Effects of Cafeteria Feeding. Cafeteria-fed animals consumed 30% more calories than did animals fed chow alone ($P < 0.001$), without gaining any more weight (103% of controls). Interscapular BAT mass was increased 2.5-fold (Table 5). In liver and epididymal fat, there were small decreases in the concentration of S14 mRNA in cafeteria-fed animals. However, in BAT, there was a doubling of this mRNA (Table 5). Cafeteria feeding decreased lipogenesis in liver, whereas in epididymal fat, levels were not different from chow-fed controls (Table 5). In BAT, ^3H incorporation into fatty acids was 2-fold higher in the animals that were overfed, matching precisely the increase seen with S14 mRNA.

Plasma T3 Levels. T3 was measured in plasma taken from 3-week cold-exposed and cafeteria-fed animals and compared to control animals (mean levels, 0.54 ng/ml). Cold exposure resulted in a small increase, which did not reach significance (126% ± 14% of controls). A larger increase was found with cafeteria feeding (156% ± 31% of controls), which was significant at the $P < 0.05$ level.

Table 3. Effect of thyroid state on lipogenesis

Tissue	Euthyroid	Hypothyroid
Liver	18.6 ± 1.7 (13)	4.8 ± 0.8* (8)
Epididymal fat	13.8 ± 2.8 (15)	5.2 ± 0.7† (8)
BAT	23.4 ± 2.8 (19)	39.3 ± 7.2† (12)

Values are mean ± SEM expressed as μmol of H incorporated per g of tissue per hr. The number of estimations is given in parentheses.
*$P < 0.001$ versus euthyroid.
†$P < 0.05$ versus euthyroid.

DISCUSSION

The levels of S14 mRNA expression in BAT are extremely high. In the fully induced rat liver this sequence accounts for ≈0.5% of the mRNA population (22). Concentrations in hypothyroid or cold-exposed BAT are 6-fold higher, implying that S14 mRNA comprises about 3% of total mRNA under these conditions. No information is available about the half-life of the corresponding protein, but the level of S14 mRNA in BAT suggests that the S14 protein may be a major constituent of BAT. BAT is a tissue that is particularly adapted for the oxidation and synthesis of fatty acids. The stimulation by cold exposure of lipolysis, β-oxidation, and the uncoupling of oxidative phosphorylation from ATP synthesis are rapid events seen within minutes and are triggered by norepinephrine release from sympathetic nerve terminals (8). However, since there are no changes in S14 mRNA for at least the first 24 hr following cold exposure, the S14 protein probably does not participate directly in these events.

It is now clear that BAT is a major site for fatty acid synthesis (23). Indeed, when fully stimulated, it is the most lipogenically active mammalian tissue yet examined (23). This is particularly interesting because BAT also contains the highest levels of S14 mRNA. Trayhurn has reported that though an hour of cold exposure does not change lipogenic activity in BAT, chronic cold dramatically stimulates lipogenesis in this tissue (24). Our results here are in good agreement; exposure for 4 hr at 5°C is without effect, but 3 weeks' exposure produces a large increase. The 9-fold stimulation seen may be in excess of the 3-fold increase in S14 mRNA observed under the same circumstances. Assuming a function for the S14 protein in the regulation of lipogenesis, this discrepancy could suggest that the cold stimulus acts at additional points in the lipogenic pathway. Alternatively, the effects of cold exposure on the S14 protein might exceed that on the mRNA, simply by increasing the stability as well as the synthesis of the protein. At this point, the mechanism whereby cold increases BAT lipogenesis remains to be elucidated.

The ability of some animals to overeat without gaining the expected amount of weight was attributed to BAT by Rothwell and Stock (10). It has been shown that feeding a cafeteria diet results in changes in BAT that are in many ways similar to those seen with cold exposure (10, 17). Trayhurn, in collaboration with Rothwell and Stock, measured lipogenesis in cafeteria-fed rats and showed that there was a 50% drop in BAT lipogenesis in comparison to controls (25). This fall was explained by the high fat composition of the diet, which diminished the need for *de novo* fatty acid synthesis to provide fuel for thermogenesis. In our hands, despite some scatter in the results, cafeteria feeding resulted in a significant doubling of fatty acid synthesis. The discrepancy between this finding and that of Trayhurn and collaborators cannot be explained at this point and may simply reflect the vagaries of cafeteria feeding. It is interesting to note that in lactating rats, Agius *et al.* found a stimulation of BAT lipogenesis with overfeeding (26).

The 2-fold stimulation of fatty acid synthesis that we observe in BAT from cafeteria-fed rats is paralleled by a

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Table 4. Effects of cold exposure

Tissue	Tissue weight, g		S14 mRNA, % standard per μg of RNA		Lipogenesis, μmol of H per g/hr	
	23°C	5°C	23°C	5°C	23°C	5°C
Liver	10.1 ± 0.6	11.4 ± 0.3*	13.4 ± 2.1 (10)	14.4 ± 1.5 (10)	19.8 ± 3.4	21.4 ± 1.5
Epididymal fat	1.55 ± 0.14	1.32 ± 0.11	91.4 ± 16.4 (12)	267.8 ± 33.4† (13)	14.9 ± 4.9	26.9 ± 6.7*
BAT	0.14 ± 0.01	0.55 ± 0.03†	225.5 ± 27.4 (12)	690.4 ± 49.1‡ (13)	26.7 ± 5.3	251.9 ± 20.7†

Animals were housed individually in the cold room or at room temperature for 3 weeks. Results are expressed as mean ± SEM of eight observations unless otherwise indicated in parentheses.
*$P < 0.05$ versus room temperature control.
†$P < 0.001$ versus room temperature control.
‡$P < 0.005$ versus room temperature control.

doubling in the concentration of S14 mRNA, preserving the link between this sequence and lipogenesis. In livers from the same animals, the two parameters are both similarly reduced, although in epididymal fat there is at least a suggestion of a dissociation. Lipogenesis rises and S14 mRNA falls, in comparison to chow-fed animals, although only the fall in S14 mRNA is significant ($P < 0.05$). Also, these chow-fed animals have elevated levels of S14 mRNA in comparison to the larger group of euthyroid animals listed in Table 1, raising the possibility that this control group is aberrant for unknown reasons. The fall in S14 mRNA induced by cafeteria feeding in liver and epididymal fat occurs despite elevated levels of plasma T3. This implies that a constituent of the diet, perhaps the high fat content, exerts an inhibitory effect on S14 mRNA in these tissues.

On the whole, in all three tissues, the level of S14 mRNA correlated well with lipogenesis when animals were subjected to the diverse stimuli of hypothyroidism, cold exposure, and cafeteria feeding. The key enzymes of lipogenesis *per se*, acetyl-coenzyme A carboxylase and fatty acid synthetase, are complex but reasonably well described (27), and it seems unlikely that S14 forms any known part of them. Nevertheless, it seems entirely possible that S14 plays some other regulatory role in facilitating lipogenesis—for example, providing substrate or reducing equivalents.

The basis for thyroid thermogenesis is poorly understood (11, 28), but evidence is mounting that the metabolism of triglycerides and fatty acids may play a major role. Not only do fatty acids constitute the fuel for increased oxygen consumption (28) but the stimulation of lipogenesis (29) and also formation of triglycerides (28) may consume significant amounts of the ATP thus generated. Indeed, as suggested by Diamant et al. (29), at least a part of the thyroid hormone-induced increase in oxygen consumption may be explained by the so-called futile cycle of synthesis and oxidation of fatty acids. However, Bartels and Sestoft (30), using perfused rat liver, showed that fractional uptake of fatty acids was not increased by thyroid hormone treatment and that a given concentration of fatty acid in the perfusate occasioned the same increase in oxygen consumption in eu- or hyperthyroid animals. They concluded that thyroid hormone did not stimulate futile cycling between fatty acid synthesis and

oxidation. In the same series of experiments they also showed that perfusion with oleate caused a greater increase in ketone body production in hyper- as opposed to euthyroid animals. It seems surprising that the increased degradation of fatty acids to ketone bodies would not be accompanied by an increase in oxygen consumption. In addition, since plasma free fatty acid levels are increased in hyperthyroidism (29), and fractional uptake remains the same (30), delivery of fatty acids to mitochondria is elevated. These fatty acids will be transported into the mitochondria for oxidation since the rate-limiting enzyme for this process, carnatine acyl transferase, is also stimulated by thyroid hormone (31).

It is informative to contrast the thermogenic mechanism in brown fat with that regulated by thyroid hormone in other tissues. BAT is unique in that it contains the mitochondrial uncoupling protein (8). Stimulation of lipolysis liberates fatty acids within the tissue that can be immediately oxidized in the mitochondria without producing ATP (8). Lipogenesis is subsequently required, simply to provide fuel for thermogenesis. Since neither lipogenesis nor, indeed, S14 mRNA rises in the short term following cold stimulation, the basal levels of fatty acid synthesis, coupled with triglyceride stores, must be sufficient to allow increased respiration for at least the first 24 hr. However, in liver and other T3-responsive tissues, increased oxygen consumption is only observed many hours after administration of thyroid hormone (32). In the absence of an uncoupling protein, major increases in respiration cannot occur without concomitant utilization of the ATP produced, since the lack of ADP will limit oxidative phosphorylation (33). Thus, thyroid-stimulated energy-consuming processes, such as lipogenesis, must be activated at least simultaneously and perhaps even as the first step in a chain of events that lead to increased oxygen consumption. Lipogenesis appears to fuel BAT and thyroid hormone-induced thermogenesis and may also have a role in stimulating the latter process by generating ADP.

A further link between thermogenesis in BAT and that due to thyroid hormone is provided by the recent demonstration from Silva and Larsen (34) that BAT possesses the type II thyroxine 5′ deiodinase enzyme. Activation of BAT by cold or norepinephrine results in stimulation of this enzyme,

Table 5. Effects of cafeteria feeding

Tissue	Tissue weight, g		S14 mRNA, % standard per μg of RNA		Lipogenesis, μmol of H per g/hr	
	Chow	Cafeteria	Chow	Cafeteria	Chow	Cafeteria
Liver	10.4 ± 0.3	9.1 ± 0.4	10.4 ± 1.5	8.5 ± 0.9	17.3 ± 2.6	10.2 ± 1.1*
Epididymal fat	1.56 ± 0.12	3.31 ± 0.22*	189.5 ± 23.0	118.0 ± 36.0†	11.5 ± 1.5	14.9 ± 2.4
BAT	0.14 ± 0.01	0.40 ± 0.04*	208.6 ± 10.2	413.5 ± 51.8‡	22.9 ± 4.1	46.1 ± 9.9†

Animals given the cafeteria diet for 2 weeks ate 130% of the calories of control fed rats ($P < 0.001$) but gained the same amount of weight (103% of controls). Values shown are mean ± SEM, $n = 8$.
*$P < 0.001$ versus chow fed.
†$P < 0.05$ versus chow fed.
‡$P < 0.005$ versus chow fed.

which leads to elevated plasma T3 levels and therefore enhanced thyroid thermogenesis.

Sundin has postulated that the hyperthyroid rat has an attenuated BAT response to cold because thyroid hormone-induced thermogenesis elsewhere diminishes the need for thermogenesis in BAT, to maintain body temperature (12). An extension of this idea may explain the elevated S14 mRNA and lipogenesis observed in these studies in hypothyroid BAT. In the hypothyroid rat, metabolism is reduced in thyroid-responsive tissues and, at temperatures below thermoneutrality, other mechanisms, perhaps in BAT, will be required to produce heat.

However, it is well known that hypothyroid rats do not tolerate cold stress (14) and that at least a part of this deficit is localized in BAT. On exposure to cold, hypothyroid rats fail to mobilize their BAT lipid stores (15), and the normal increase in GDP binding to mitochondria, indicative of increased uncoupled respiration, is not seen (13). The number of β-adrenergic receptors is diminished in hypothyroid BAT, and, in addition, there appear to be postreceptor defects (35). Despite this blocked BAT response to cold, there is evidence that this tissue may still be more thermogenically active in the unstressed hypo- as opposed to euthyroid rat. Thus, hypothyroid BAT exhibits some changes that are similar to its response to cold, including increases in oxygen consumption (15) and GDP binding to mitochondria (13).

The mechanism whereby BAT S14 mRNA, lipogenesis, or, indeed, thermogenesis is stimulated in the hypothyroid state is unknown. A likely possibility is that it is indirect, with a drop in body temperature in the hypothyroid state being monitored centrally and then communicated to BAT by way of sympathetic nerve stimulation. However, the presence of T3 receptors in BAT raises the possibility of a direct, suppressive effect of the hormone. Whatever the mechanism, there must be important differences in the regulatory elements surrounding the S14 gene in brown fat as opposed to liver, which permit this differential regulation.

Overall it seems reasonable to suppose that an animal's thermogenic requirements can be met by a variety of mechanisms, of which that localized in BAT is one and that induced by thyroid hormones is another. Given the same thermogenic requirements, a change in the amount of heat supplied by thyroidal mechanisms will result in an opposite and compensatory change in BAT metabolism. The S14 protein, acting at some point in the production pathway for fatty acids, may be crucial in both processes.

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