Enhanced expression of uncoupling protein 2 gene in rat white adipose tissue and skeletal muscle following chronic treatment with thyroid hormone

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Abstract Evidence is rapidly emerging which suggests that uncoupling protein 2 (UCP2), by virtue of its ubiquitous expression, may be important for determining basal metabolic rate. To assess the functional modulation of UCP2 gene expression in relation to body weight control, we examined the effects of hyperthyroid state induced by chronic treatment with triiodothyronine (T_3) on UCP2 mRNA expression in male rats. Daily subcutaneous injection of T_3 (37 pmol/100 g body weight) for 7 days increased UCP2 mRNA expression in brown adipose tissue (BAT), white adipose tissue (WAT) and the soleus muscle 1.6-, 1.6- and 1.7-fold compared to the controls, respectively, and increased UCP1 mRNA expression in BAT 1.2-fold. In contrast, the same treatment with T_3 decreased both ob mRNA expression in WAT and plasma leptin level 0.5-fold for each. The present results suggest that T_3 may directly increase UCP2 expression independently of leptin action.

Key words: Uncoupling protein 2; Hyperthyroidism; Leptin; Adipose tissue; Skeletal muscle

1. Introduction

Uncoupling protein (UCP) is a unique protein located in the inner mitochondrial membrane and its main function is to allow proton re-entry into the mitochondrial matrix [1]. This proton dissipates energy as heat instead of being used for adenosine triphosphate production [1]. It is well known that UCP plays an important role in the regulation of energy expenditure in mammals [2].

UCP1, by virtue of its unique expression in brown adipose tissue (BAT) and its cyclic adenosine monophosphate-responsive element, primarily mediates cold exposure-induced thermogenesis for the purpose of defending body temperature. This thermogenesis due to UCP1 is controlled by activity of efferent sympathetic nerves to β_3-adrenergic receptors (β_3-AR) of BAT [3,4]. There is a growing body of evidence that thyroid hormone is one of the essential factors affecting UCP1 activity. Sensitivity of β_3-AR and expression of UCP1 mRNA in response to cold were lower in hypothyroid rats [5]. These defects were fully corrected by replacement of triiodothyronine (T_3) [5]. UCP1 mRNA in BAT of rat fetuses with hyperthyroidism increased 1.2-fold compared to euthyroid controls [6]. Thus, UCP1 in BAT is believed to be a major thermogenic effector and its activity is modulated at least in part by thyroid hormone in rodents [7]. In adult humans, however, the limited BAT cannot explain the increased energy expenditure during hyperthyroidism attributable to enhanced expression of UCP1 mRNA. BAT in humans proliferates to express UCP1 [8] only under conditions of prolonged cold exposure or catecholamine producing tumors.

A novel member of the UCP family, termed UCP2, has been cloned recently [9]. Evidence to date indicates that the product of the UCP2 gene is crucial for mammalian thermogenesis similarly to UCP1 because of its high degree of sequence similarity (55–60%) to UCP1 [9]. UCP2 is widely expressed in human and rodent tissues [9–12], unlike UCP1, which is expressed uniquely in BAT. The ubiquitous expression of UCP2 suggests that the protein may be important for determining basal metabolic rate, and possibly regulating body weight in mammals including humans. Thyroid hormone is known to increase the basal metabolic rate in human [13]. Taken together, these findings led to a hypothesis based on possible mechanisms that increased basal metabolic rate during hyperthyroidism may depend on increases not only in UCP1 but also in UCP2 mRNA expression. Therefore, thyroid hormone may regulate the expression of both proteins.

To test this hypothesis, hyperthyroid rats treated with chronic administration of T_3 were examined for UCP2 mRNA expression in BAT, white adipose tissue (WAT) and skeletal muscle. Since expression of UCP2 mRNA is directly enhanced by plasma leptin concentration [14], expression of the ob gene and plasma leptin levels were also examined. Possible interactions of thyroid hormone with UCP2 expression and leptin secretion are discussed.

2. Materials and methods

2.1. Experimental procedure

Mature male Wistar King A rats (Seac Yoshitomi Ltd., Fukuoka, Japan), 259–275 g at 8–9 weeks of age, were used. They were housed in a soundproof room illuminated daily from 7.00 to 19.00 h (a 12:12 h light:dark cycle) with temperature at 21 ± 1°C, and humidity 55 ± 5%. The rats were allowed free access to tap water and standard pellet rat chow (CE-2, CLEA Japan Ltd., Tokyo, Japan). Body weight was measured daily at 9.00 h. Matched on the basis of body weight on the first injection day, eight rats were equally divided into T_3-treated (T_3) and saline-treated control groups. The solution of T_3 (Aldrich Chemical Co., Milwaukee, USA) was freshly dissolved in physiological saline at a concentration of 0.37 mM. To increase plasma T_3 level

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Abbreviations: UCP, uncoupling protein; WAT, white adipose tissue; BAT, brown adipose tissue; β_3-AR, β_3-adrenergic receptors

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and render animals hyperthyroid, rats in the T_3 group were daily injected subcutaneously with T_3 solution at a dose of 37 pmol/100 g body weight in a volume of 0.1 ml solution for 7 days. The injection procedure of the controls was same as that of the T_3 group. The T_3 group was deprived of T_3 treatment for 24 h before blood sampling. After decapitation, BAT, the epididymal fat tissue (WAT) and the soleus muscle were surgically removed and immediately frozen in liquid nitrogen, then stored at −80°C until RNA extraction.

2.2. Measurement of plasma T_3, T_4, TSH and leptin concentration

Plasma samples were collected at 12.00 h immediately before and on the 7th day after the treatment following an overnight fast. The sample was immediately frozen at −20°C. T_3, thyroxine (T_4) and thyroid stimulating hormone (TSH) were measured with a commercially available RIA kit (T_3 and TSH RIA beads, Dainabot, Tokyo, Japan; M-T_4 RIA Chiron 2, Chiron, California, USA). Plasma leptin was measured with a commercially available kit (sandwich enzyme immunoassay, Immune Biological Laboratory, Gunma, Japan). The lowest limit of detection for leptin was 200 pg/ml.

2.3. Northern blot analysis

Total cellular RNAs were prepared from various rat tissues with the use of Isogen (Nippon Gene, Toyama, Japan) according to the manufacturer's protocol. Total RNAs (20 μg/lane) of samples from the BAT, WAT and the skeletal muscle were electrophoresed on 1.2% formaldehyde-agarose gel and the separated RNAs were transferred onto a Biodyne B membrane (Pall Canada Ltd., Mississauga, Ont., Canada) in 20×SSC by capillary blotting [15] and immobilized by exposure to ultraviolet light (0.80 J). Prehybridization and hybridization were carried out according to the method described by Yang et al. [16], except that ^{32}P-labeled rat UCP2 cDNA fragment was used as a probe (unpublished data). Membranes were washed under high stringency conditions [16]. After washing the membranes, the hybridization signals were analyzed with a BIO-image analyzer BAS 2000 (Fuji Film Institution, Tokyo, Japan). The membranes were stripped by exposure to boiling 0.1% SDS and rehybridized with a ribosomal RNA (rRNA) that was used to quantify the amounts of RNA species on the blots.

All data are expressed as the mean ± S.D. The statistical significance of differences was assessed by the unpaired *t*-test. The animals were used in accordance with the Oita Medical University Guidelines based on the NIH Guide for the Care and Use of Laboratory Animals.

3. Results

3.1. Effects of chronic T_3 treatment on plasma concentrations of T_3, T_4, TSH and body weight

Table 1 shows changes in plasma levels of T_3, T_4 and TSH, and body weight after daily T_3 injection for 7 days. Plasma T_3 level significantly increased in the T_3 group compared with that in the control group ($P < 0.001$). Plasma T_4 level decreased more in the T_3 group than in the controls ($P < 0.001$). TSH in these groups became undetectable after 7-day treatment with T_3. The body weight before the treatment did not differ between the two groups. Body weight in the T_3 group decreased after the 7-day T_3 treatment, in contrast to the increase in body weight of the controls during the same period.

3.2. Effects of chronic T_3 treatment on UCP2 mRNA expression in BAT, WAT and skeletal muscle, and on UCP1 mRNA expression in BAT

Northern blot analyses of UCP2 mRNA in BAT, WAT and the skeletal muscle, and of UCP1 mRNA in BAT are shown in Fig. 1. The T_3 treatment increased UCP2 mRNA expression in all those tissues 1.6-, 1.6-, and 1.7-fold, respectively, compared to the controls ($P < 0.001$ for each). UCP2 mRNA in the heart also increased 2.3-fold in the T_3 group (0.05 ± 0.002, mRNA/rRNA a.u.) compared to the controls (0.022 ± 0.006, mRNA/rRNA a.u.; $P < 0.001$ vs. T_3 group) (data not shown in Fig. 1). The expression of UCP1 mRNA in BAT increased 1.2-fold in the T_3 group compared with to the controls ($P < 0.01$).

3.3. Effects of chronic T_3 treatment on ob mRNA in WAT and plasma leptin concentration

As shown in Fig. 2A, chronic T_3 treatment decreased ob mRNA expression in WAT 0.5-fold compared with the controls ($P < 0.001$). Plasma leptin concentration concomitantly decreased 0.5-fold in the T_3 group, compared to the controls ($P < 0.001$) (Fig. 2B).

4. Discussion

The present study demonstrates that hyperthyroid state induced by 7-day treatment with T_3 increased rat mRNA expression of not only BAT UCP1 but also UCP2 in BAT, WAT, skeletal muscle and the heart. These findings raise a question as to whether the enhanced expression of UCP2 induced by chronic treatment with T_3 may be the result of a direct or an indirect effect. It is suggested that there are possible interactions between T_3 and leptin and sympathetic nerve activity.

Overexpression of leptin per se has been shown to increase UCP2 mRNA in lean Zucker rats (+/+) [14]. If leptin is a mediator of UCP2 under the hyperthyroidism in the present study, T_3 treatment should up-regulate ob gene expression. In contrast, the present data show decreases in ob gene expression and plasma leptin concentration. This decrease is consistent with the previous finding that prolonged administration of T_3 decreased body weight and fat mass, resulting in a reduction of leptin secretion [17]. Together with the data presented here, the foregoing findings seem to demonstrate that leptin is not the mediator of T_3-induced increase in UCP2 expression.

BAT is known to be richly innervated by sympathetic efferent nerves. Hyperactivity of sympathetic nerves in hyperthyroidism enhances UCP1 expression in BAT. In this context, it is postulated that β_3-adrenergic activity in hyperthyroidism may be one of the UCP2 regulators similar to UCP1 in hyper-

Table 1
Changes in plasma concentration of T_3, T_4 and TSH and body weight before and after chronic treatment with T_3

	n	T_3 (ng/ml)	T_4 (ng/ml)	TSH (μU/ml)	Body weight (g)	
					Before	After
Controls	4	0.5 ± 0.04	3.0 ± 0.7	ND	263.3 ± 4.9	274.8 ± 3.8
T_3-treated	4	7.0 ± 0.9**	1.0 > **	ND	265.6 ± 7.6	247.1 ± 8.0†

Values are the mean ± S.D. of *n* treated rats. ND, not detectable. Before/after, before/after the 7-day T_3 treatment. T_3-treated, the group injected with daily subcutaneous T_3 at a dose of 37 mmol/100 g body weight.
†$P < 0.05$ vs. the corresponding initial value. **$P < 0.001$ vs. the corresponding controls.

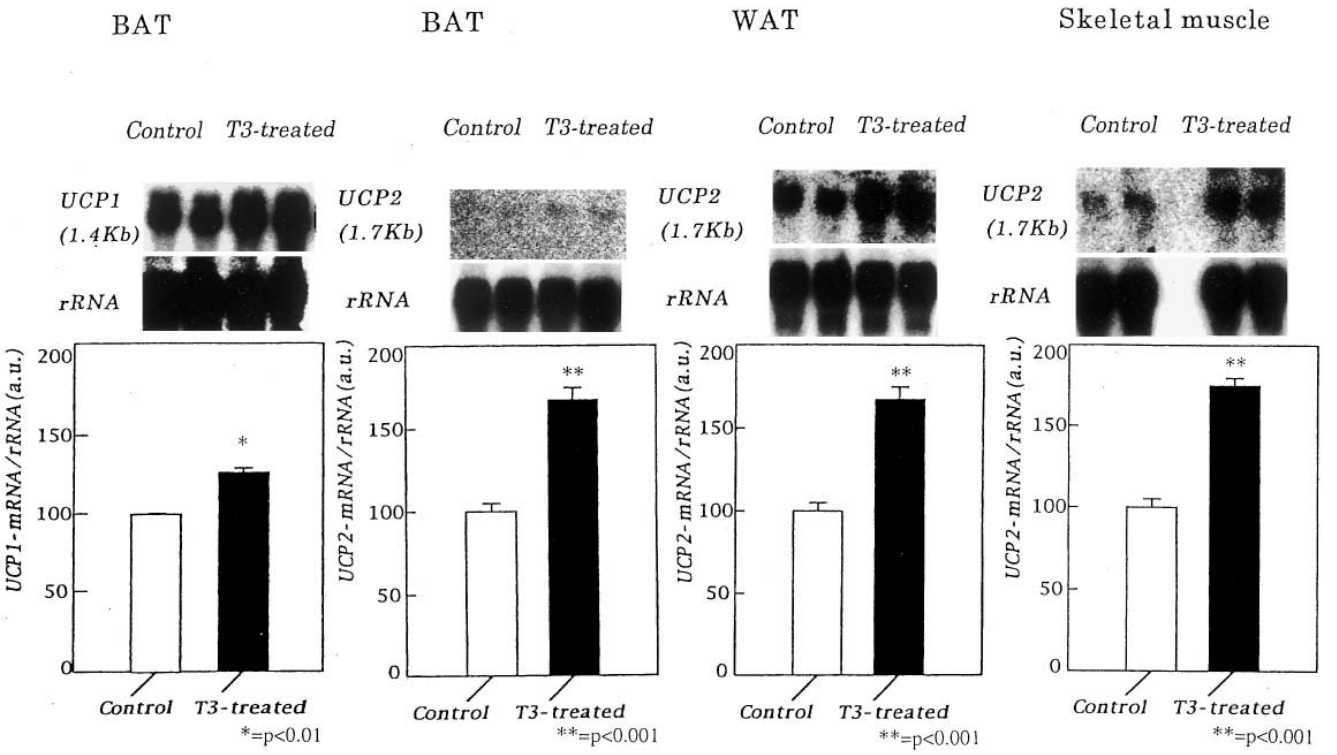

Fig. 1. The effects of T_3 on UCP2 mRNA expression in the BAT, WAT and skeletal muscle, and on UCP1 mRNA expression in BAT. Northern blot analysis of UCP2 was performed in total RNA (20 μg/lane) of the samples from BAT, WAT and skeletal muscle, and that of UCP1 from BAT. To quantify amounts of mRNAs from those tissues, the blots were reprobed with rRNA. Values and vertical bars, mean ± S.D. ($n = 4$). a.u., arbitrary units. $^{**}P < 0.001$, $^{*}P < 0.01$ vs. the corresponding controls.

thyroidism. Evidence to date, however, shows that this is still not clear, because expression of UCP2 can be significantly changed in catecholamine-null mice [18], and the effect of β_3-adrenergic activity on UCP2 expression is reported inconsistently by investigators [9,19].

A recent study has demonstrated that fasting increases UCP2 mRNA expression in skeletal muscle [19]. In the present study, hyperthyroidism reduced body weight and increased UCP2 expression in skeletal muscle. These findings indicate the possibility that body weight loss may cause the elevation of UCP2 expression. However, the assumption that the elevated UCP2 expression may be caused by the weight loss per se can be negated for the following reasons. (1) Body weight loss due to fasting did not affect UCP2 expression in the heart or BAT [19]. Thus, weight reduction does not necessarily reflect changes in UCP2 mRNA expression in all bod-

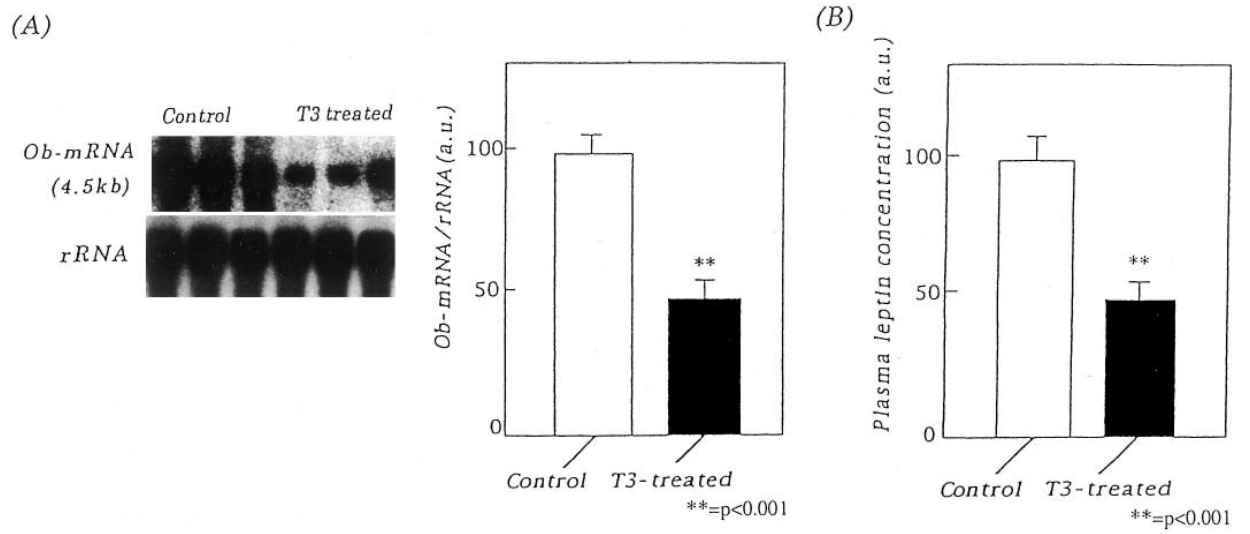

Fig. 2. The effects of T_3 on ob gene mRNA expression in the WAT (A) and plasma leptin concentration (B). Northern blot analysis of ob gene was performed in total RNAs (20 μg/lane) of WAT samples. To quantify amounts of mRNA from WAT, the blots were reprobed with rRNA. Values and vertical bars, mean ± S.D. ($n = 4$). a.u., arbitrary units. $^{**}P < 0.001$, $^{*}P < 0.01$ vs. the control.

ily tissues. (2) In the present study, body weight reduction following hyperthyroidism increased UCP2 expression even in the heart and BAT.

Evidence to date substantially suggests that T_3 may directly up-regulate UCP2 mRNA expression in BAT, WAT, skeletal muscle and the heart. The present study provides evidence that thyroid hormone is a potent activator of UCP2. The relation between thyroid hormone and UCP2 is indispensable for understanding the physiological regulation mechanisms of energy balance and metabolism. The present results may help to explain the elevated energy expenditure in hyperthyroidism.

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