

## ORIGINAL ARTICLE

# Comprehensive study of urinary cortisol metabolites in hyperthyroid and hypothyroid patients

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## Summary

**Objective** To further analyse the significance and mutual relationship of thyroid function-linked alterations in cortisol metabolism that have been separately and variously reported.

**Patients and measurements** Twenty-four-hour urine samples from 21 patients with hyperthyroidism (Graves' disease), 16 patients with hypothyroidism (Hashimoto's thyroiditis), 21 healthy age- and sex-matched controls for hyperthyroidism, and 16 healthy age- and sex-matched controls for hypothyroidism were evaluated for 6 $\beta$ -hydroxycortisol (6 $\beta$ -OHF), tetrahydrocortisol (THF), tetrahydrocortisone (THE), allo-tetrahydrocortisol (allo-THF), urinary free cortisol (UFF), urinary free cortisone (UFE) and 17-hydroxycorticosteroid (17-OHCS).

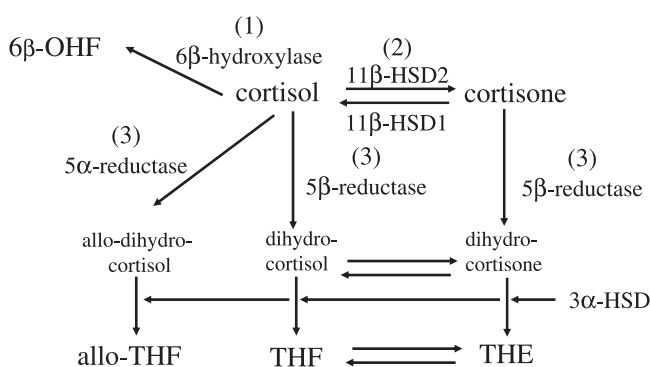
**Results** Urinary 17-OHCS, THE and allo-THF levels increased considerably in hyperthyroid patients compared to the controls, while UFF and THF showed no difference between the two groups. Urinary 6 $\beta$ -OHF was significantly lower in the hyperthyroid patients than in the controls. Both the urinary allo-THF + THF/THE and the UFF/UFE ratios were significantly lower in the hyperthyroid patients than in the controls, whereas only the former was significantly higher in the hypothyroid patients than in the controls. The urinary allo-THF/THF ratio was significantly higher in the hyperthyroid patients and significantly lower in the hypothyroid patients than in the controls. In an analysis of pooled subjects including all groups ( $n = 64$ ), free T4 levels correlated negatively ( $P < 0.0001$ ) with the urinary allo-THF + THF/THE ratio but not with the UFF/UFE ratio. The serum levels of free T4 correlated positively ( $P < 0.0001$ ) with the urinary allo-THF/THF ratio.

**Conclusion** The thyroid hormones seem to affect the total 11 $\beta$ -HSD activity (allo-THF + THF/THE) more strongly than the renal 11 $\beta$ -HSD2 activity (UFF/UFE). 5 $\alpha$ -reductase activity (allo-THF/THF) is also enhanced in hyperthyroidism, while the reduction of urinary 6 $\beta$ -OHF in hyperthyroidism might be a secondary effect of the altered activity of the total 11 $\beta$ -HSD and 5 $\alpha$ -reductase.

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## Introduction

The general metabolic pathways of cortisol are shown in Fig. 1. Cortisol is reduced to allo-dihydrocortisol (allo-DHF) and DHF by 5 $\alpha$ - and 5 $\beta$ -reductase, respectively, and then to allo-THF and THF, respectively.<sup>1,2</sup> Cortisol is linked to cortisone by 11 $\beta$ -HSD, which is known to consist of two subunit isozymes. 11 $\beta$ -HSD type 1 (11 $\beta$ -HSD1) is a bidirectional enzyme that acts as both a dehydrogenase (cortisol to cortisone) and a reductase (cortisone to cortisol). The reductase activity is thought to be dominant *in vivo*. The enzyme is expressed along with the glucocorticoid receptor (GR), and thereby regulates glucocorticoid (GC) activity. Substantial amounts of 11 $\beta$ -HSD1 are found in the human liver, adipose tissue, gonad, brain, pituitary and vascular smooth muscle.<sup>3–5</sup> 11 $\beta$ -HSD type 2 (11 $\beta$ -HSD2) is found in the same organs as the mineralocorticoid receptor (MR). 11 $\beta$ -HSD2 manifests only dehydrogenase action (cortisol-to-



(1) The urinary 6 $\beta$ -OHF/creatinine ratio was used as an index for 6 $\beta$ -hydroxylase activity.

(2) The urinary allo-THF + THF/THE ratio was used as an index for overall 11 $\beta$ -HSD activity.

(3) The urinary allo-THF/THF ratio was used as an index for 5 $\alpha$ -reductase/5 $\beta$ -reductase activity.

Fig. 1 Metabolic pathways of cortisol, as revealed by urinary metabolites.

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cortisone conversion), which either inhibits the binding of GC to MR or preserves the selectivity of aldosterone activity at the MR. This second enzyme responsible for cortisol metabolism is found in the kidney, colon, gastrointestinal tract and salivary gland.<sup>3,5-7</sup> There are two types of 5 $\alpha$ -reductase:<sup>1,12,17</sup> 5 $\alpha$ -reductase type 1 is expressed in the liver and skin after puberty, while 5 $\alpha$ -reductase type 2 is localized in the prostate and epididymis. Both convert testosterone into dihydrotestosterone (DHT), the strongest androgen in the body.<sup>18</sup> The enzyme 6 $\beta$ -hydroxylase is also responsible for cortisol metabolism, albeit only for a very small part of it (an estimated 1% of the overall cortisol metabolism).<sup>1</sup> Through this pathway, cortisol is excreted into the urine in the form of 6 $\beta$ -hydroxycortisol (6 $\beta$ -OHF), a metabolite also used as an index of the activity of cytochrome P450 (CYP) 3A4 in the liver.<sup>4,10</sup> In addition, some cortisol (F) and cortisone (E) is excreted into the urine without prior metabolism. Urinary 17-hydroxycorticosteroid (17-OHCS) has long been known to reflect all the fractions of the corticosteroids, possessing a hydroxyl group at position 17 of the steroid structure; that is, THE, THE, allo-THE, 6 $\beta$ -OHF, F and E.<sup>11</sup> Reports on hyperthyroid subjects have described an enhanced reduction of the A-ring double bond of the steroid structure (C4–5) and increased 11 $\beta$ -HSD activity.<sup>3-9,12</sup> Several earlier reports have also asserted that altered thyroid states influence urinary 6 $\beta$ -OHF.<sup>11,13,14</sup> The aim of the present study was to clarify the mutual relationships between these cortisol metabolites through extensive measurement of various metabolites found in urine specimens collected over a 24-h period from patients with hyperthyroidism or hypothyroidism.

## Subjects and methods

### Subjects

Twenty-one hyperthyroid patients (16 women and five men) and 16 hypothyroid patients (12 women and four men) were selected from the outpatient and inpatient clinics of our department and enrolled in this study together with 27 healthy controls (19 women and eight

men). For both the hyper- and the hypothyroid groups, sex-, age- and body mass index (BMI)-matched controls were selected: 21 controls (16 women and five men) for the hyperthyroid group and 16 controls (12 women and four men) for the hypothyroid group. The hyperthyroid cases were all diagnosed with Graves' disease. Nine were on antithyroid drugs and the other 12 received no medication. The nine medicated patients received thiamazole at a dose of either 30 mg/day ( $n = 4$ ) or 15 mg/day ( $n = 5$ ). Hyperthyroidism was diagnosed in these patients based on the free T4, free T3 and TSH levels. All 16 of the hypothyroid patients were diagnosed with Hashimoto's thyroiditis. Eight were on thyroid hormone replacement treatment while the other eight remained untreated. The eight medicated patients received levothyroxine sodium at a dose of 12.5  $\mu$ g/day ( $n = 2$ ), 50  $\mu$ g/day ( $n = 3$ ) or 100  $\mu$ g/day ( $n = 3$ ). Hypothyroidism was diagnosed in these patients based on the free T4, free T3 and TSH levels. The 27 healthy controls consisted of medical staff with blood concentrations of the thyroid hormones (free T4 and free T3) and TSH within normal ranges. None of them took any drugs capable of inhibiting or inducing CYP3A, an enzyme responsible for 6 $\beta$ -OHF generation.

The hyper- and hypothyroid patients were matched with healthy controls for age, sex and BMI for comparison. The blood concentrations of the thyroid hormones were significantly higher in the hyperthyroid patients and significantly lower in the hypothyroid patients than in their age- and sex-matched controls (Table 1).

This study was approved by the ethics committee of the Kinki University School of Medicine. Written informed consent was obtained from all subjects.

### Methods

Urine was collected for 24 h from hyperthyroid patients, hypothyroid patients, and healthy controls. The urine samples were stored frozen at  $-20^{\circ}\text{C}$  until assay.

Urinary THE, THE and allo-THE were measured on a chromatographic system (LC-MS/MS) composed of a liquid chromatograph

**Table 1.** Subject characteristics at baseline

	N (F/M)	Age (years)	BMI (kg/m <sup>2</sup> )	TSH ( $\mu$ IU/ml)	FT4 (pmol/l)	FT3 (pmol/l)	Creatinine (g/day)	Urine volume (ml)
Controls	21 (16/5)	37 $\pm$ 17 (F: 35 $\pm$ 16) (M: 43 $\pm$ 25)	21.7 $\pm$ 2.4	2.21 $\pm$ 1.10	17.3 $\pm$ 2.8	5.2 $\pm$ 0.9	1.23 $\pm$ 0.85	1239 $\pm$ 579
Hyperthyroid patients	21 (16/5)	40 $\pm$ 15 (F: 37 $\pm$ 14) (M: 47 $\pm$ 18)	21.6 $\pm$ 1.4	< 0.1**	60.32 $\pm$ 6.7**	18.12 $\pm$ 0.2**	0.98 $\pm$ 0.29	1108 $\pm$ 397
Controls	16 (12/4)	50 $\pm$ 20 (F: 46 $\pm$ 18) (M: 70 $\pm$ 8)	22.8 $\pm$ 2.3	2.74 $\pm$ 0.96	16.9 $\pm$ 2.0	4.9 $\pm$ 0.4	1.1 $\pm$ 0.58	1430 $\pm$ 425
Hypothyroid patients	16 (12/4)	55 $\pm$ 12 (F: 52 $\pm$ 10) (M: 64 $\pm$ 14)	23.2 $\pm$ 4.5	123.30 $\pm$ 120.71*	5.0 $\pm$ 3.4**	1.8 $\pm$ 1.2**	0.95 $\pm$ 0.28	1209 $\pm$ 378

The data are expressed as mean  $\pm$  SD. M, male; F, female; BMI, body mass index; FT4, free T4; FT3, free T3.

\* $P < 0.01$ , \*\* $P < 0.0001$  vs. controls.

(Agilent, CA, USA) and a mass chromatograph (Micromass, Ltd, Great Dunmon, UK). Each aliquot (0.1 ml) of the sampled urine was hydrolysed for 2 h at 50 °C with 0.2 ml of an acetic acid buffer containing  $\beta$ -glucuronidase (4200 U/ml;  $\beta$ -G, 2100 U/ml; sulfatase). After hydrolysis, the sample was mixed for 5 min with 0.1 ml of an internal standard (6 $\alpha$ -methylprednisolone, 13  $\mu$ mol/l) and 0.6 ml of methanol, and then centrifuged at about 11 000 g for 5 min. The resulting supernatant was diluted fivefold with purified water and then analysed on an LC-MS/MS system composed of an MS/MS Quattro-Ultima connected to an HP 1100 Series LC.

Urinary 17-OHCS was measured using an OH-Kit (Kanto Kagaku, Tokyo, Japan), which uses a colorimetric method (Porter-Silber reaction), in accordance with the manufacturer's instructions.

Urinary free cortisol (UFF) was measured by high-performance liquid chromatography (HPLC) on a Shimadzu LC-10 A series chromatographer. Five ml of ethyl acetate was added to 1 ml of the sampled urine and mixed for 15 s or more. The mixture was centrifuged at about 2000 g for 5 min and the organic layer was extracted. Two ml of a 1 mol/l sodium hydroxide solution (including 20% sodium sulfate) was added to the organic layer, mixed for 15 s, and centrifuged at about 2000 g. The organic layer was extracted, concentrated at 40 °C under a nitrogen air current, dried to a solid, and redissolved in 0.3 ml of a 10 mmol potassium dihydrogen phosphate/acetonitril (130 : 7) solution. The solution was subjected to HPLC.

Urinary free cortisone (UFE) was measured by HPLC on a Shimadzu LC-10 A series chromatographer. A small volume (0.1 ml) of acetonitril was added to 0.4 ml of the sampled urine and mixed for 5 min using a mixer. After centrifugation at about 2000 g for 5 min, the supernatant was removed and measured by HPLC.

Urinary 6 $\beta$ -OHF was measured using an enzyme-linked immunosorbent assay (ELISA) kit (Stabiligen, Nancy, France), in accordance with the manufacturer's instructions.

Androsterone and etiocholanolone levels were measured for some subjects (10 hyperthyroid patients and 10 controls) using 17-KS fractionation [gas chromatography mass spectroscopy (GC-MS)] comprising the following steps: (1) hydrolysis of the urine specimen with  $\beta$ -glucuronidase and sulfatase, (2) extraction with dichloroethane, (3) condensation of the extracted layer, (4) trimethylsilylation, (5) fractionation and (6) determination on GC-MS.

The methods described above were deemed suitable for use in this study after a preliminary study confirmed the reproducibility of the measurement of the metabolites investigated. The urinary accumulation procedures used for the measurement of the urinary metabolites were also judged to be suitable, based on a preliminary investigation of the urinary volume and creatinine concentrations in the collected urine samples measured using a Shikari Kit-S CRE (Kanto Kagaku, Tokyo, Japan).

The measured levels of urinary 17-OHCS, UFF, 6 $\beta$ -OHF, THF, THE and allo-THF were calibrated with the creatinine levels from the same specimen and expressed as grams (g)/grams of creatinine (g crn).

In the present study, the enzyme activities in cortisol metabolism were indirectly estimated from the ratios of the urinary metabolites, as follows. Urinary 6 $\beta$ -OHF was used as the index of 6 $\beta$ -hydroxylase activity.<sup>10,15</sup> The urinary allo-THF + THF/THE ratio was used as the index of the overall 11 $\beta$ -HSD set-point.<sup>7-10,17,20</sup> The urinary allo-

THF/THF ratio was used as the index of the relative activities of 5 $\alpha$ -reductase/5 $\beta$ -reductase.<sup>24,25</sup> In addition, as an alternative to the serum DHT/testosterone ratio as a 5 $\alpha$ -reductase activity indicator, the ratio of androsterone to etiocholanolone, which is a urinary metabolite of androstenedione, was used.<sup>26</sup> The UFF/UFE ratio was used as the index of renal 11 $\beta$ -HSD2 activity, although its significance is still controversial.<sup>7-9,19-21</sup>

The levels of TSH, free T4 and free T3 were measured using the Architect™ TSH, FT4 and FT3 kits (Abbott Japan Co., Ltd, Tokyo, Japan), respectively, with the chemiluminescent immunoassay (CLIA) method.

### Statistical analysis

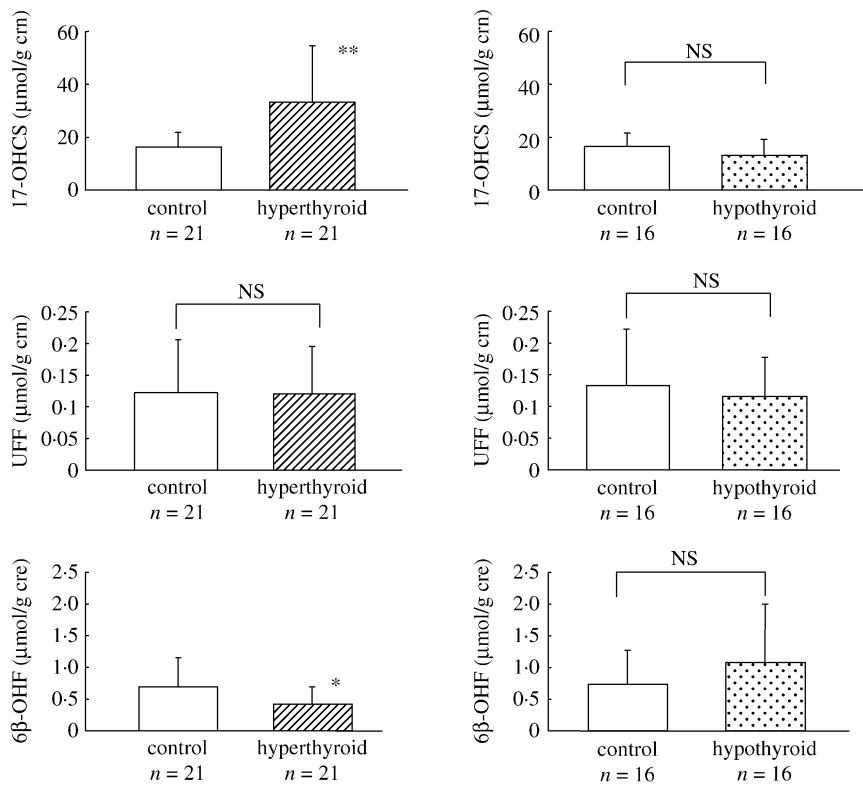
The data obtained were expressed as means  $\pm$  standard deviation and assessed for significant differences using Student's unpaired *t*-test. Correlations were calculated using the least-squares method.  $P < 0.05$  was considered statistically significant. The statistical analyses were performed using StatView 5.0 for Windows (SAS Institute, Inc., Cary, NC, USA).

### Results

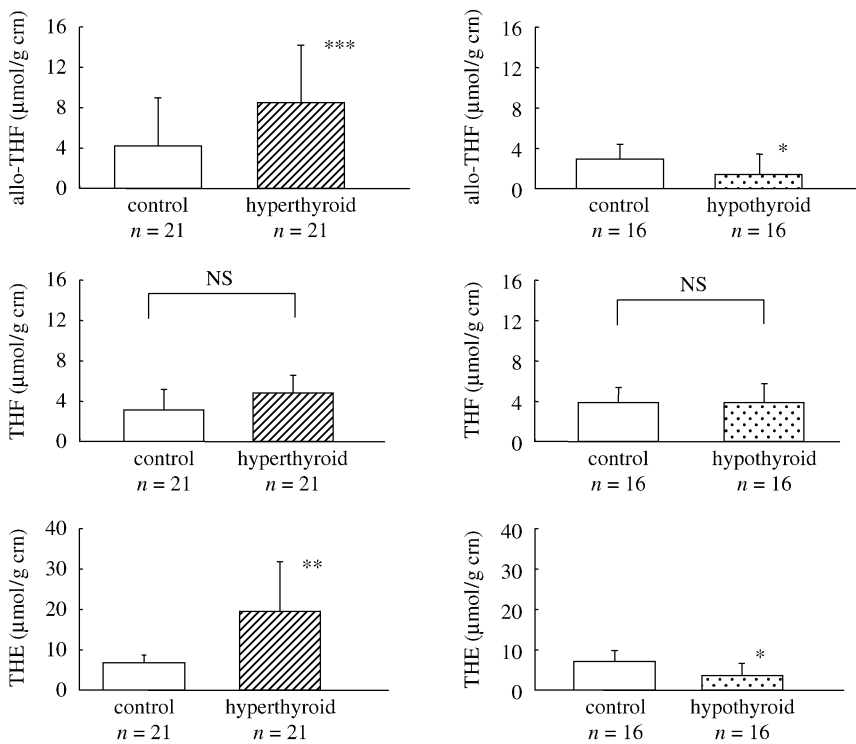
The urinary amounts of 17-OHCS ( $\mu$ mol/g crn) were significantly higher in hyperthyroid patients than in the controls ( $P = 0.0004$ ), whereas they did not significantly differ between the hypothyroid patients and the controls (Fig. 2, upper panels). However, the UFF levels ( $\mu$ mol/g crn) were not significantly higher than the control levels in either patient group (Fig. 2, middle panels). The UFE levels ( $\mu$ mol/g crn) did not differ significantly from the control levels in either the hyperthyroid or the hypothyroid group (data not shown). The urinary 6 $\beta$ -OHF level was significantly lower than the control level in the hyperthyroid patients and higher than the control level in the hypothyroid patients, although not to a statistically significant degree (Fig. 2, lower panels).

Figure 3 shows the data on urinary allo-THF, THF and THE separately for hyper- and hypothyroid patients. The allo-THF and THE levels were significantly higher than the control levels in the hyperthyroid patients ( $P < 0.0001$ ) and significantly lower than the control levels in the hypothyroid patients (Fig. 3, upper and lower panels). However, the THF levels in the hyper- and hypothyroid patients were not significantly different from the control levels (Fig. 3, middle panels). These findings are basically in agreement with a study conducted by Hellman *et al.*<sup>22</sup> on the metabolic transformation of tracer doses of radioactive hydrocortisone given to six hyperthyroid patients. However, thyroid function test results were not available and age- and sex-matching were not performed in their study.

The urinary allo-THF + THF/THE ratio, an estimate of overall 11 $\beta$ -HSD, was significantly lower in the hyperthyroid group ( $P = 0.002$ ) and significantly higher in the hypothyroid group ( $P = 0.01$ ) than the control levels (Fig. 4, upper panels). The ratio of urinary free cortisol to cortisone (UFF/UFE) has been tentatively postulated to serve as a specific index of renal 11 $\beta$ -HSD type 2 activity, although its true utility remains to be established.<sup>7-9,16,19,21</sup> This ratio was significantly lower in hyperthyroid patients than in the controls



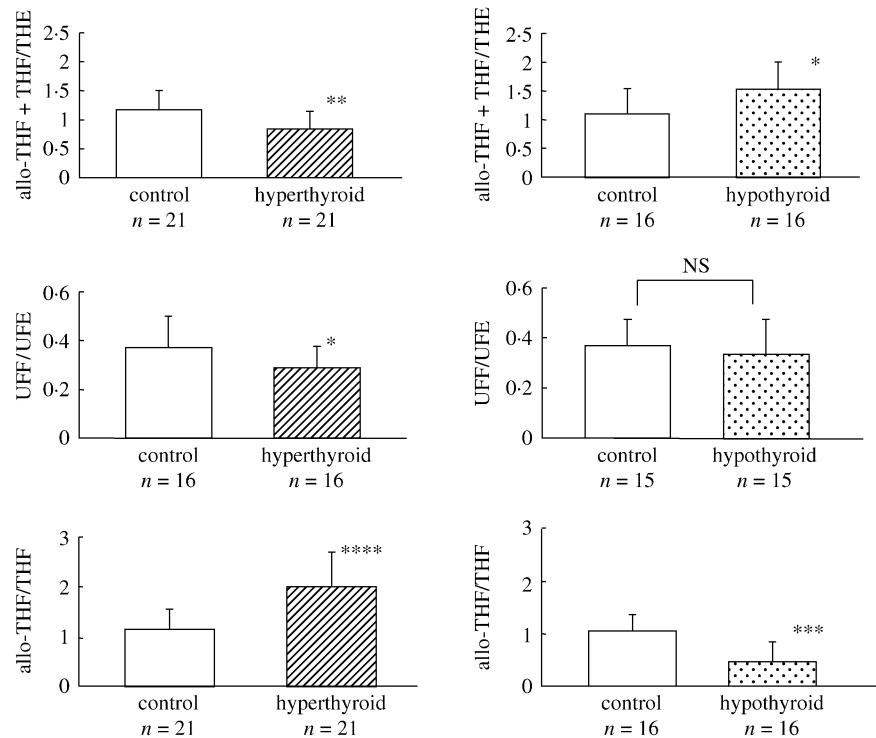
**Fig. 2** Urinary 17-OHCS, UFF and 6β-OHF in hyperthyroid and hypothyroid patients. The data are expressed as means ± SD. \**P* < 0.05; \*\**P* < 0.001; NS, not significant compared with the controls.



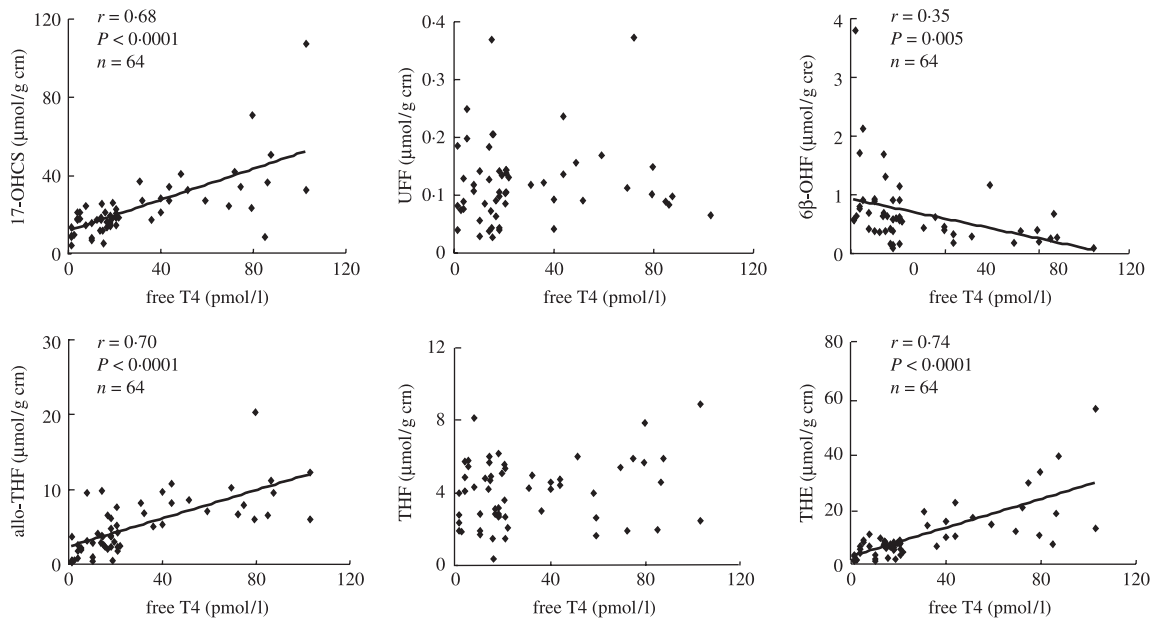
**Fig. 3** Urinary allo-THF, THF and THE in hyperthyroid and hypothyroid patients. The data are expressed as means ± SD. \**P* < 0.05; \*\**P* < 0.001; \*\*\**P* < 0.0001; NS, not significant compared with the controls.

(*P* = 0.03) and did not differ significantly between the hypothyroid patients and controls (Fig. 4, middle panels). The urinary allo-THF/THF ratio, which is the index of the relative activities of 5α-reductase/5β-reductase, was significantly higher in the hyperthyroid group

(*P* < 0.0001) and significantly lower in the hypothyroid group (*P* = 0.0007) than the control ratio (Fig. 4, lower panels). In a separate comparison of the androsterone/etiocolanalone ratio between the 10 hyperthyroid patients (six women and four men) and 10 controls



**Fig. 4** Urinary allo-THF + THF/THE, UFF/UFE and allo-THF/THF ratios in hyperthyroid and hypothyroid patients. The data are expressed as means  $\pm$  SD. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; \*\*\*\* $P < 0.0001$  compared with the controls. NS, not significant compared with the controls.



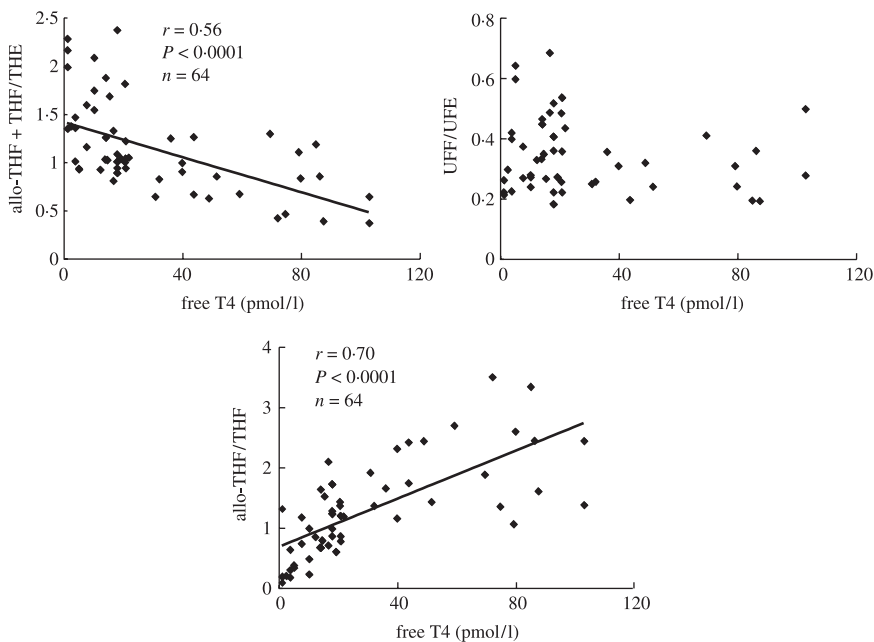
**Fig. 5** Relationship between serum free T4 and urinary 17-OHCS, UFF, urinary 6β-OHF, allo-THF, THF and THE in all subjects (hyperthyroid,  $n = 21$ ; hypothyroid,  $n = 16$ ; control,  $n = 27$ ).

(six women and four men), the ratio was significantly higher in the former ( $2.97 \pm 1.29$  vs.  $1.63 \pm 0.82$ , mean  $\pm$  SD) (data not shown in Fig. 4).

When the correlation between the cortisol metabolites in urine and serum free T4 was investigated in the entire study population (21 hyperthyroid patients, 16 hypothyroid patients and 27 controls), urinary 17-OHCS, allo-THF and THE showed strong positive

correlations with serum free T4 ( $r = 0.68$ – $0.74$ ,  $P < 0.0001$ ), while urinary 6β-OHF showed a somewhat weaker inverse correlation with serum free T4 ( $r = 0.35$ ,  $P = 0.005$ ), as shown in Fig. 5.

Figure 6 shows the respective relationships of the urinary allo-THF + THF/THE, UFF/UFE and allo-THF/THF ratios with the serum free T4 levels in the overall study population. Allo-THF + THF/THE correlated negatively with free T4 levels ( $P < 0.0001$ ),



**Fig. 6** Relationship between serum free T4 and urinary allo-THF + THF/THE, UFF/UFE and allo-THF/THF in all subjects (hyperthyroid,  $n = 21$ ; hypothyroid,  $n = 16$ ; control,  $n = 27$ ).

while no correlation was found between the UFF/UFE ratio and the serum free T4 levels. Allo-THF/THF correlated positively with free T4 levels ( $P < 0.0001$ ) (Fig. 6, lower panel).

We examined how the variations in the urinary cortisol metabolite levels obtained in this study were related to each other in hyperthyroid patients (data not shown). The proportion of all 17-OHCS metabolites put together was 87–90% in the controls, 98% in the hyperthyroid patients, and 73% in the hypothyroid patients, indicating that the marked elevations in urinary THE and allo-THF in the hyperthyroid patients were clearly responsible for the elevation in the overall 17-OHCS level.

Finally, we examined the effects of antithyroid drugs and thyroid hormone medication used to treat thyroid conditions on the present results. The Graves' disease group and the Hashimoto's thyroiditis group were each divided into two subgroups, one subgroup comprising those taking oral antithyroid drugs or oral thyroid hormones and the other subgroup comprising those receiving no medication. To ensure that the effects of the therapeutic agents are eliminated, these two subgroups were further divided into mild and severe cases, based on the hormone level (mild, FT4  $\leq 5.1$  pmol/l for Graves' disease and FT4  $> 5.1$  pmol/l for Hashimoto's thyroiditis; severe, FT4  $> 5.1$  pmol/l for Graves' disease and FT4  $\leq 5.1$  pmol/l for Hashimoto's thyroiditis). The mild and severe cases receiving oral agents were matched for age, sex and BMI with those receiving no medication. As shown in Table 2, no significant differences were found between the medicated and the nonmedicated groups in the ratios of urinary 6 $\beta$ -OHE, 17-OHCS, allo-THF + THF/THE and allo-THF/THF in either the mild or the severe cases.

## Discussion

We have extensively measured urinary cortisol metabolites, including 17-OHCS, free cortisol, 6 $\beta$ -OHE, allo-THF, THF and THE, in the same samples obtained from patients with thyroid disease and from

controls. In this well-controlled study, the 17-OHCS level in the hyperthyroid patients was found to be markedly higher than that in the controls, while that in the hypothyroid group was not significantly different from the control level. Although these results partly agree with earlier reports,<sup>23</sup> the absence of any significant difference between the control and the hypothyroid group in our study was discrepant. That is, the enhanced action of the thyroid hormones themselves was confirmed to be a leading factor behind the increase in urinary 17-OHCS, and the converse of this was not evident in the hypothyroid group. In an attempt to identify which metabolite elicited the increase in urinary 17-OHCS in the hyperthyroid patients, we found that the increase in both THE and allo-THF was responsible. This was inconsistent with the findings of Hellman *et al.*,<sup>22</sup> who reported a smaller increase of allo-THF in hyperthyroidism and attributed it to a secondary result caused by the marked THE increase leading to the overproduction of cortisol. In our study, however, both allo-THF and THE increased significantly in association with a positive correlation with free T4 (Fig. 3), while THF remained unchanged in hyperthyroidism compared to the levels in controls. It thus appears that the thyroid hormone had relatively direct effects on the activities of 11 $\beta$ -HSD and 5 $\alpha$ -reductase. In addition, UFF (a reflection of cortisol itself) did not change in the hyperthyroid patients. This finding indicates that the cortisol metabolism, in particular that of THE and allo-THF, increases in hyperthyroidism, leading to the dynamic homeostasis of the absolute serum cortisol level, which naturally requires increased cortisol production to maintain the ACTH–cortisol axis in its normal state.

Although the low urinary allo-THF + THF/THE ratio, reflecting the overall activity of 11 $\beta$ -HSD, found in the hyperthyroid patients can be attributed to a marked increase in THE, it seems to result from both the suppressed cortisone (E)-to-cortisol (F) conversion (11 $\beta$ -HSD1) and the increased F-to-E conversion (11 $\beta$ -HSD2). Several recent reports<sup>7–10,20</sup> have contended that the UFF/UFE ratio is superior to the allo-THF + THF/THE ratio as an index of 11 $\beta$ -HSD2



**Table 2.** Comparison of urinary cortisol metabolites in hyperthyroid and hypothyroid patients with or without treatment

	N (F/M)	Age (years)	BMI (kg/m <sup>2</sup> )	TSH ( $\mu$ IU/ml)	FT4 (pmol/l)	FT3 (pmol/l)	6 $\beta$ -OHF ( $\mu$ mol/g crn)	17-OHCS ( $\mu$ mol/g crn)	allo-THF + THF/THE	allo-THF/THF
<i>Hyperthyroid</i>										
Controls	21 (16/5)	37 $\pm$ 17	21.7 $\pm$ 2.3	2.21 $\pm$ 1.05	17.2 $\pm$ 2.8	5.2 $\pm$ 0.9	0.69 $\pm$ 0.46	18.1 $\pm$ 4.9	1.17 $\pm$ 0.33	1.13 $\pm$ 0.44
Treatment (–) FT4 $\leq$ 51 (pmol/l)	5 (4/1)	40 $\pm$ 14	21.1 $\pm$ 1.4	< 0.1	39.8 $\pm$ 6.9	12.1 $\pm$ 3.3	0.51 $\pm$ 0.25 NS	30.2 $\pm$ 10.3*** NS	0.84 $\pm$ 0.27 NS	1.84 $\pm$ 0.52** NS
Treatment (+) FT4 $\leq$ 51 (pmol/l)	5 (4/1)	50 $\pm$ 10	22.0 $\pm$ 1.4	< 0.1	41.9 $\pm$ 7.0	9.4 $\pm$ 2.2	0.37 $\pm$ 0.07 NS	29.1 $\pm$ 2.3*** NS	1.02 $\pm$ 0.22 NS	1.65 $\pm$ 0.40* NS
Treatment (–) FT4 > 51 (pmol/l)	7 (5/2)	38 $\pm$ 11	22.8 $\pm$ 1.6	< 0.1	89.2 $\pm$ 14.8	25.7 $\pm$ 9.0	0.49 $\pm$ 0.37 NS	45.7 $\pm$ 27.7*** NS	0.64 $\pm$ 0.27** NS	2.16 $\pm$ 0.85*** NS
Treatment (+) FT4 > 51 (pmol/l)	4 (3/1)	30 $\pm$ 11	21.1 $\pm$ 1.5	< 0.1	77.2 $\pm$ 6.7	25.2 $\pm$ 6.3	0.41 $\pm$ 0.24 NS	34.5 $\pm$ 26.4** NS	0.81 $\pm$ 0.29* NS	2.29 $\pm$ 0.87*** NS
<i>Hypothyroid</i>										
Controls	16 (12/4)	50 $\pm$ 20	22.8 $\pm$ 2.1	2.73 $\pm$ 0.86	16.9 $\pm$ 2.0	4.9 $\pm$ 0.4	0.74 $\pm$ 0.53	17.5 $\pm$ 5.0	1.17 $\pm$ 0.43	1.00 $\pm$ 0.35
Treatment (–) FT4 > 5.1 (pmol/l)	5 (3/2)	63 $\pm$ 8	22.2 $\pm$ 1.6	38.1 $\pm$ 36.4	9.0 $\pm$ 2.3	2.7 $\pm$ 0.7	1.14 $\pm$ 0.88 NS	15.3 $\pm$ 6.9 NS	1.41 $\pm$ 0.42 NS	0.61 $\pm$ 0.32 NS
Treatment (+) FT4 > 5.1 (pmol/l)	4 (3/1)	58 $\pm$ 18	23.8 $\pm$ 4.1	22.2 $\pm$ 17.9	8.0 $\pm$ 1.6	2.9 $\pm$ 0.4	0.68 $\pm$ 0.26 NS	15.8 $\pm$ 6.7 NS	1.60 $\pm$ 0.38 NS	0.67 $\pm$ 0.35 NS
Treatment (–) FT4 $\leq$ 5.1 (pmol/l)	3 (2/1)	64 $\pm$ 13	21.3 $\pm$ 2.7	177.8 $\pm$ 134.6	2.3 $\pm$ 1.9	1.3 $\pm$ 1.2	1.24 $\pm$ 1.39 NS	11.3 $\pm$ 5.9 NS	1.59 $\pm$ 0.54 NS	0.45 $\pm$ 0.50* NS
Treatment (+) FT4 $\leq$ 5.1 (pmol/l)	4 (4/0)	60 $\pm$ 12	23.8 $\pm$ 4.1	122.2 $\pm$ 90.2	3.9 $\pm$ 1.1	1.5 $\pm$ 0.8	0.97 $\pm$ 0.50 NS	16.6 $\pm$ 4.8 NS	1.30 $\pm$ 0.20 NS	0.32 $\pm$ 0.21** NS

Hyperthyroid patients were treated using antithyroid drugs and hypothyroid patients were treated using thyroid hormone.

The data are expressed as mean  $\pm$  SD. F, female; M, male; BMI, body mass index; FT4, free T4; FT3, free T3; NS, not significant.

\* $P$  < 0.05, \*\* $P$  < 0.01, \*\*\* $P$  < 0.001 vs. controls.

levels in the kidney. We investigated the same issue with the present data. Our results revealed that the UFF/UFE ratio was significantly lower than the control level only in the hyperthyroid patients, whereas the ratio in the hypothyroid patients was not different from the control ratio. There was no correlation between UFF/UFE and serum thyroid hormone levels. Therefore, compared to the results for allo-THF + THF/THE, the relationship between UFF/UFE and the thyroid hormones was weak. Obviously, more detailed studies will be required to elucidate the specific significance of allo-THF + THF/THE and UFF/UFE.

The activities of 11 $\beta$ -HSD1 and 11 $\beta$ -HSD2 were separately measured in the colons and kidneys of rats with thyroxine-induced hyperthyroidism. The 11 $\beta$ -HSD1 activity in the colons and kidneys of the hyperthyroid rats did not differ from that in the same organs of euthyroid control rats, whereas the 11 $\beta$ -HSD2 activity was significantly higher in the colons, but not in the kidneys, of the hyperthyroid rats than in the controls.<sup>3</sup> In a study using hyperthyroid rats, Tenore *et al.*<sup>26</sup> have shown that the mucosa-to-serosa Cl<sup>-</sup> transport decreased and the Cl<sup>-</sup>/HCO<sub>3</sub><sup>-</sup> anion exchange was inhibited. These changes apparently influenced the transepithelial flux transport and intestinal motility. The increase of 11 $\beta$ -HSD2 in the colon could thus be the mechanism behind the effects of thyroxine on the Cl<sup>-</sup>/HCO<sub>3</sub><sup>-</sup> anion exchange and on the contractility of the colon, which usually increases in thyrotoxic patients.<sup>3,27,28</sup> Ricketts *et al.*<sup>30</sup> have examined the regulation of 11 $\beta$ -HSD1 using primary cultures of rat and human hepatocytes. The thyroid hormones increased 11 $\beta$ -HSD1 activity in rat hepatocytes but had no effect on 11 $\beta$ -HSD1 activity in human hepatocytes. Tomlinson *et al.*<sup>19</sup> have reviewed studies analysing the regulation of 11 $\beta$ -HSD1 in a number of tissues from different species. The thyroid hormones were found to increase 11 $\beta$ -HSD1 expression in rat hepatocytes (*in vitro*), whereas they inhibited 11 $\beta$ -HSD1 expression in the rat liver (*in vivo*) but not in human hepatocytes (*in vitro*).<sup>30</sup> No report has yet verified this phenomenon in humans (*in vivo*). Accordingly, our present data may indirectly support the notion that 11 $\beta$ -HSD2 activity is enhanced in organs other than the kidney in hyperthyroid patients, as has been suggested by the notable elevation of the urinary allo-THF + THF/THE ratio as compared to the UFF/UFE ratio. While *in vivo* reductase activity is dominant over dehydrogenase activity in 11 $\beta$ -HSD1, 11 $\beta$ -HSD1 possesses both reductase activity and dehydrogenase activity. The relative increase in the dehydrogenase activity of 11 $\beta$ -HSD in hyperthyroidism may be partly due to the enhancement of dehydrogenase activity of 11 $\beta$ -HSD1 as well as an increase in the amount of 11 $\beta$ -HSD2.

The present study revealed a strong correlation between the urinary allo-THF/THF ratio, reflecting the relative activities of 5 $\alpha$ -reductase/5 $\beta$ -reductase, and the thyroid hormones. The ratio was significantly higher in the hyperthyroid patients and significantly lower in the hypothyroid patients than in the controls. In addition, the androsterone/etiocholanolone ratio was significantly higher in the hyperthyroid group than in the controls. When urinary allo-THF and THF were separately studied in relation to the blood concentrations of the thyroid hormones, only the former showed a strong positive correlation with free T4. The urinary allo-THF levels were also significantly higher in the hyperthyroid group and significantly lower in the hypothyroid than in the controls, whereas the urinary

THF levels did not differ from the control levels in either the hyperthyroid or the hypothyroid group. Thus, our results seem to have uncovered a specific thyroid hormone-induced enhancement of 5 $\alpha$ -reductase activity. Vantghem *et al.*<sup>12</sup> have previously reported that the allo-THF/THF ratio decreased in cases of overt hypothyroidism, possibly in association with a disturbance of 5 $\beta$ -reductase activity. Ram *et al.*<sup>29</sup> have demonstrated a thyroxine-induced increase in 5 $\alpha$ -reductase activity and 5 $\alpha$ -reductase mRNA in rats. In our data using the allo-THF/THF ratio and in our comparative evaluation of individual THF and allo-THF data, the predominance of 5 $\alpha$ -reductase in hyperthyroidism is clear. Therefore, we speculate that the thyroid hormones may directly affect 5 $\alpha$ -reductase activity.

In our present study of 6 $\beta$ -hydroxylase activity (estimated by measurements of 6 $\beta$ -OHF), the effects of the thyroid hormones were found to be inhibitory. That is, the urinary 6 $\beta$ -OHF/creatinine ( $\mu$ mol/g crn) levels were significantly lower in the hyperthyroid patients than in the controls and higher in the hypothyroid patients than in the controls, although the differences were not significant. These results support earlier data reported by Yamaji *et al.*<sup>11</sup> and Yamada.<sup>14</sup> In the investigation of all groups (hyperthyroid patients, hypothyroid patients and controls), the urinary 6 $\beta$ -OHF and serum thyroid hormone levels showed a weak inverse correlation. However, when the relationship between the thyroid hormones and urinary 6 $\beta$ -OHF was investigated separately in the hyper- and hypothyroid groups, no correlation was found between free T4 and 6 $\beta$ -OHF in either group. This indicates that the thyroid hormones are unlikely to influence 6 $\beta$ -OHF levels directly. Rather, the metabolic elevations of THE and allo-THF in hyperthyroidism may cause a secondary decline in urinary 6 $\beta$ -OHF. However, given that 6 $\beta$ -OHF is an index of 6 $\beta$ -hydroxylase activity, careful future observation will be required to determine precisely how the reduction in enzyme activity in hyperthyroid patients leads to delayed drug metabolism.

To summarize, we have examined the role of altered cortisol-metabolizing enzyme activity in altered thyroid states in humans by determining the major urinary metabolites of cortisol. We found, as a result, that some pathways, especially those involving 5 $\alpha$ -reductase and 11 $\beta$ -HSD, are directly influenced by the thyroid hormones, while the 6 $\beta$ -hydroxylase pathway is indirectly affected. This issue should be further studied in detail through the selective detection of enzyme activity at the histological level in each organ.

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