Metabolic Clearance and Secretion Rates of Subunits of Human Thyrotropin

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ABSTRACT Metabolic clearance rates (MCR) of the alpha and beta subunits of human thyrotropin (hTSH-α and hTSH-β) were determined by a constant infusion to equilibrium method. In 15 normal individuals (six men, six premenopausal women, and three postmenopausal women), the mean MCR of hTSH-α (68 ml/min per m^2) was significantly faster than that of hTSH-β (48 ml/min per m^2) was significantly faster than that of hTSH-β (48 ml/min per m^2); both were two to three times more rapid than the previously determined MCR of hTSH. In patients with primary hypothyroidism, MCR were significantly slower with a mean value of 55 ml/min per m^2 for hTSH-α and 37 ml/min per m^2 for hTSH-β. However, MCR of subunits were not significantly faster than normal in hyperthyroid patients.

Serum concentrations of alpha subunits and hTSH-β were measured by radioimmunoassay, and secretion rates of alpha and hTSH-β from the pituitary were calculated using hTSH-α and hTSH-β MCR, respectively. In the normal individuals, alpha secretion rates averaged 91 μg/day per m^2, greater than those previously determined for hTSH and human follicle-stimulating hormone. Alpha secretion rates were significantly elevated in the normal postmenopausal women (211 μg/day per m^2) and in the premenopausal hypothyroid women (202 μg/day per m^2); they were also elevated in the postmenopausal hypothyroid women (277 μg/day per m^2). Alpha secretion rates were significantly decreased in the premenopausal hyperthyroid women (66 μg/day per m^2). Usually, the secretion rates of hTSH-β could not be calculated in normal individuals, and the rates in hyperthyroid patients could never be calculated because serum hTSH-β was not detected. Six normals had detectable hTSH-β secretion rates (17 μg/day per m^2); hTSH-β secretion rates were significantly increased in patients with primary hypothyroidism (28 μg/day per m^2). Although we had previously demonstrated a 50-fold increase in hTSH secretion rates in primary hypothyroidism, there was only a 2-fold increase in alpha and hTSH-β secretion rates. Thus, increased subunit synthesis appears to be utilized predominantly for production of complete hTSH.

INTRODUCTION

The development of sensitive and specific radioimmunoassays (1–4) for the immunologically indistinguishable alpha subunits (α)[1] of the human glycoprotein hormones, thyrotropin (hTSH), follicle-stimulating hormone (hFSH), luteinizing hormone (hLH), and chorionic gonadotropin (hCG), and for the beta subunit of hTSH (hTSH-β), has permitted measurement of subunits in biological fluids. We have previously reported that both the alpha subunit and hTSH-β are present in the serum of patients with primary hypothyroidism and are secreted from the pituitary

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[1] *Abbreviations used in this paper*: alpha or α, immunologically common alpha subunit of the glycoprotein hormones; fT_4, free thyroxine; hCG, human chorionic gonadotropin; hCG-α, alpha subunit of hCG; hFSH, human follicle-stimulating hormone; hLH, human luteinizing hormone; hTSH, human thyrotropin; hTSH-α, alpha subunit of hTSH; hTSH-β, beta subunit of hTSH; *Kav*, partition coefficient; LHRH, luteinizing hormone-releasing hormone; MCR, metabolic clearance rates; *r*, correlation coefficient; TRH, thyrotropin-releasing hormone; T_3, total triiodothyronine by radioimmunoassay; T_4; total thyroxine by competitive protein binding assay; Vo, void volume; Vt, total bed volume.

after the administration of thyrotropin-releasing hormone (TRH) (2, 5). In addition, normal postmenopausal women have higher serum alpha concentrations than premenopausal women or men (2, 6); furthermore, serum alpha concentrations in normal individuals demonstrate greater increases after luteinizing hormone-releasing hormone (LHRH) than after TRH (7–10).

In previous studies, we have determined the metabolic clearance rates (MCR) and production rates of hTSH in various disorders of thyroid function and found that changes in the serum concentration of hTSH are mainly due to altered pituitary hTSH secretion rather than to changes in hTSH MCR (11). In the present study, we have determined the MCR of hTSH-α and hTSH-β in normal individuals and in patients with thyroid disorders. We have also measured endogenous alpha and hTSH-β serum concentrations by radioimmunoassay and calculated the pituitary secretion rate of subunits (12). Thus, we have been able to compare changes in pituitary secretion rate of hTSH to that of hTSH subunits in various states of thyroid dysfunction.

METHODS

Determination of MCR of subunits. hTSH-α was a gift of Dr. C. H. Li, University of California, San Francisco, Calif.; hTSH-β was obtained from Calbiochem, San Diego, Calif.; and hCG-α was a gift of Dr. O. Bahl, State University of New York at Buffalo, Buffalo, N.Y. Immunological and physicochemical testing showed that these subunits had properties indistinguishable from those previously prepared by our laboratory (1). Each subunit was labeled with either ^{125}I or ^{131}I to a specific activity of 50–80 μCi/μg by a modification (13) of the chloramine-T method (14) utilizing one to three additions of 180 ng of chloramine-T without sodium metabisulfite. Labeled subunit was separated from aggregated products of iodination and unreacted iodine by gel chromatography on a 1.5 × 90-cm column of Sephadex G-100 (Pharmacia Fine Chemicals Inc., Piscataway, N. J.). Further preparation of labeled subunit for infusion was identical to that previously described for hTSH (11). 70–75 ml of infusate containing a mixture of labeled subunits, 0.15 M sodium chloride, and 1.0% human serum albumin was injected, 20–25 ml being given initially as a bolus to achieve equilibrium more rapidly. After 15 min, the remainder was infused at a constant rate (0.15–0.17 ml/min) for 4–5 h. Apparent equilibrium was defined as that time after which subsequent measurements of immunoprecipitable labeled subunit levels showed no variation that exceeded ±10% of the mean immunoprecipitable labeled subunit concentration during the preceding 1-h period (11, 15). After infusion to equilibrium, labeled subunit was separated from serum or infusate by a double antibody technique with either excess rabbit anti-hTSH-α or anti-hTSH-β at a final concentration of 1:1,000. At the time of infusion, 70–90% of each tracer was immunoprecipitable. Each subject received hTSH-α labeled with ^{125}I or ^{131}I; hTSH-β labeled with ^{125}I or ^{131}I was separately infused 4–7 days before or after the infusion of hTSH-α. Both isotopes were used to label each subunit in separate experiments, and no significant differences between isotopes were noted. The total radioactivity administered to a single subject was 50 μCi or less. The measurement of serum immunoprecipitable counts at equilibrium and the calculation of MCR were performed in the same fashion as previously described for hTSH (11).

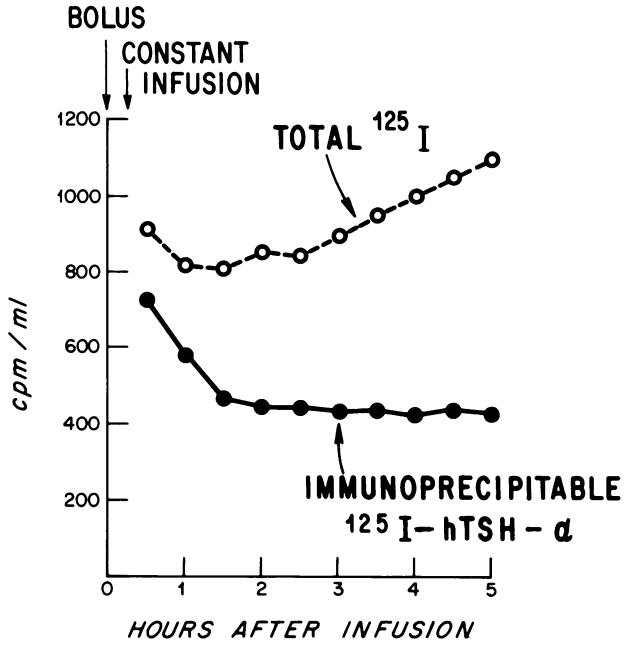

FIGURE 1 Total ^{125}I and immunoprecipitable ^{125}I-hTSH-α during infusion of ^{125}I-hTSH-α to a representative normal individual. Apparent equilibrium of immunoprecipitable counts per minute per milliliter was achieved by 3 h, whereas total ^{125}I cpm/ml continued to increase with time. In data not shown, at all time points precipitability of labeled subunit with phosphotungstic acid was greater than with antiserum to alpha subunit. Therefore, the increase in total ^{125}I represented both deiodination of labeled subunit and formation of nonimmunoreactive species.

In several subjects, a blood sample was obtained 30 min after beginning constant infusion of ^{125}I-hTSH-α or ^{131}I-hTSH-α and separate infusion of ^{125}I-hTSH-β or ^{131}I-hTSH-β; a 2-ml aliquot of this serum was co-chromatographed with free ^{125}I (to determine the total bed volume of the column, Vt) on a 1.5 × 86-cm column of Sephadex G-100. The void volume of the column, Vo, was determined with ^{125}I-bovine thyroglobulin in separate experiments. 2-ml fractions were collected and counted. All peaks of radioactivity were tested for immunoprecipitability with either excess anti-hTSH-α or anti-hTSH-β.

Radioimmunoassays and other assays. All radioimmunoassays were performed using a double antibody technique. The immunoassays of hTSH (16), hFSH (17), hLH (17, 18), and total triiodothyronine (T_3) (19) were performed by our previously published methods. The methodology and specificity of the subunit immunoassays have also previously been reported (1, 2, 5). In the current studies, the sensitivity of the hTSH-β immunoassay was increased to 0.02 ng/ml in the assay tube or 0.2 ng/ml in serum by a 48-h delay in addition of tracer to other assay reagents. Measurement of total thyroxine (T_4) was by competitive protein displacement assay and free thyroxine (fT_4) by equilibrium dialysis (16).

Subjects and experimental protocol. 15 normal in-

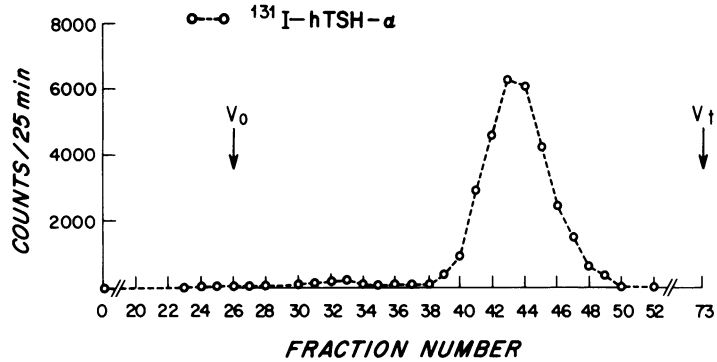

FIGURE 2 Fractionation by gel chromatography on a Sephadex G-100 column of a 2-ml aliquot of serum obtained 30 min after beginning constant infusion of ^{131}I-hTSH-α. Monomeric ^{131}I-hTSH-α had an elution volume (*Kav*, 0.36) similar to that of standard unlabeled alpha subunit (*Kav*, 0.35). Identical technique was used for fractionation of ^{125}I-hTSH-α, ^{125}I-hTSH-β, and ^{131}I-hTSH-β, which had been separately infused into other individuals. The elution volumes of labeled hTSH-β (*Kav*, 0.44) were identical to that of standard unlabeled hTSH-β (1). Small amounts of larger molecular weight aggregates (peak for ^{131}I-hTSH-α at fraction 33) were nonimmunoreactive (see text) and, therefore, were not used in calculation of MCR.

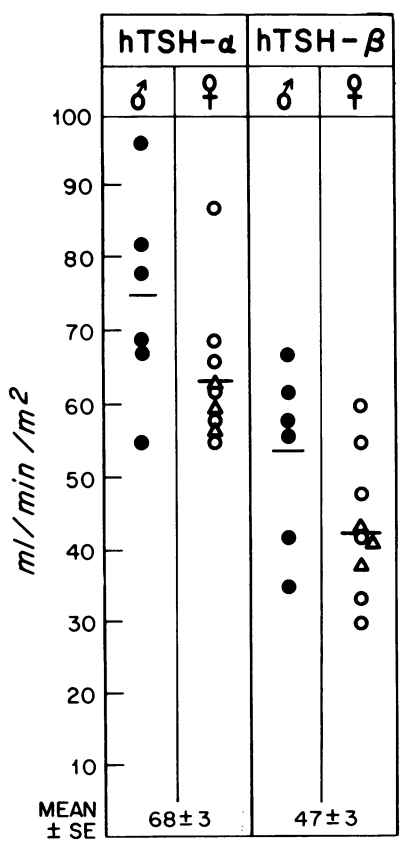

FIGURE 3 MCR (milliliters per minute per square meters) of hTSH-α and hTSH-β in 15 normal subujects (6 men [●], 6 premenopausal women [○], and 3 postmenopausal women [△]. MCR (mean±SE) for each subunit in the 15 normal subjects is shown at the bottom of the figure.

dividuals, 6 men (ages 18–60 yr; mean T_4, 6.5 μg/dl; fT_4, 1.3 ng/dl; and T_3 113 ng/dl), 6 premenopausal women with normal menstrual cycles (ages 21–30 yr; mean T_4, 6.7 μg/dl; fT_4, 1.3 ng/dl; and T_3, 91 ng/dl), and 3 postmenopausal women (ages 50–58 yr; mean T_4 6.7 μg/dl; fT_4, 1.1 ng/dl; and T_3, 76 ng/dl) were studied. In addition, eight women with primary hypothyroidism, four premenopausal and four postmenopausal, participated in the study; each had elevated serum hTSH (18–164 μU/ml), low T_4 (0.5–3.5 μg/dl), and fT_4 (0.1–0.6 ng/dl), and, all except one, low T_3 (10–60 ng/dl). Two patients with central (pituitary or hypothalamic) hypothyroidism and inappropriately low gonadotropins, one of whom had a pituitary tumor, were also studied. Finally, five women (three premenopausal and two postmenopausal) with hyperthyroidism, four with a diffuse goiter and one with an autonomous nodule, were included in the study. Each had undetectable serum hTSH (<0.5 μU/ml), elevated T_3 (290–540 ng/dl), and, all except one, elevated T_4 (13.5–19 μg/dl) and fT_4 (3.0–5.4 ng/dl). No subject was taking any medication, and all gave informed consent to participate in this study.

All subjects received an i. v. 200-μg bolus of TRH (Abbott Laboratories, North Chicago, Ill.) and 200 μg of LHRH (Ayerst Laboratories, New York) with a 4–7-day interval between tests; serum samples were collected at various times over a 180-min period for measurement of alpha, hTSH-β, hTSH, hLH, hFSH, T_4, fT_4, and T_3. MCR of hTSH-α and hTSH-β were separately determined with a 4–7-day interval between tests (see above); all euthyroid patients received 270 mg of potassium iodide immediately before infusion of labeled subunits. No change in T_4, fT_4, T_3, or hTSH was noted between the beginning and end of the determination of each MCR. All 15 normal subjects also had MCR of hCG-α determined simultaneously with hTSH-α; one subunit was labeled with ^{125}I or ^{131}I, and the other was labeled with the opposite isotope. In several of the study subjects, serum samples were also collected for measurement of alpha at 30-min intervals for 4½ h. In six other normal subjects, two men, two premenopausal women, and two postmenopausal women, serum alpha was measured at 20-min intervals for 1 day. (These samples were kindly supplied

by Dr. Robert Boyar, University of Texas Southwestern Medical School, Dallas, Tex.).

Calculation of endogenous secretion rates. The secretion rate of alpha subunits (μg/day per m^2) was calculated as the product of the endogenous serum concentration of alpha (ng/ml) and the MCR of hTSH-α (ml/min per m^2) times 1,440 min/day. Secretion rate of hTSH-β was similarly calculated utilizing serum concentration of hTSH-β and MCR of hTSH-β.

RESULTS

MCR of hTSH-α and hTSH-β. Validity of the determined MCR of these subunits depended on iodinated subunit concentrations being at equilibrium in serum. Apparent equilibrium was indeed achieved between 2½ and 3 h after the beginning of infusion in all subjects since there was no variation of immunoprecipitable counts after that time that exceeded ±10% of the mean counts in at least three serum samples drawn during the preceding 1-h period (Fig. 1). Fractionation by gel chromatography on a Sephadex G-100 column of serum obtained 30 min after the start of constant infusion in several patients revealed that small quantities of larger molecular weight aggregates of iodinated subunits were present. However, these aggregates were not immunoprecipitable (<1%), and, thus, only the immunoreactive monomeric forms were included in the calculation of MCR (Fig. 2). In addition, monomeric hTSH-α, labeled with either ^{125}I or ^{131}I, had a partition coefficient (*Kav*) of 0.36, similar to that of standard unlabeled alpha subunits; likewise, monomeric hTSH-β labeled either with ^{125}I or ^{131}I had *Kav* identical to each other and to standard unlabeled hTSH-β (*Kav*, 0.44).

There were no significant differences in MCR of hTSH-α and hTSH-β between the six normal premenopausal women and six normal men when the clearance rates were expressed in terms of body surface area (Fig. 3 and Table I), as we have previously reported for MCR of hTSH (11). These 12 normal subjects had a mean MCR of hTSH-α of 70±4 ml/min per m^2, which was significantly faster than the mean hTSH-β

TABLE I
Endocrine Data

Patient group	No. of patients	hTSH	hLH	hFSH	Alpha	MCR hTSH-α	Secretion rate, Alpha	Peak alpha post-TRH	Peak alpha post-LHRH	hTSH-β	MCR hTSH-β	Secretion rate, hTSH-β	Peak hTSH-β post-TRH
		μU/ml	mIU/ml		ng/ml	ml/min/m^2	μg/day/m^2	ng/ml		ng/ml	ml/min/m^2	μg/day/m^2	ng/ml
Normal		(0.5–3.2)	(6–26)‡	(5–25)‡	(0.5–2.0)‖					(<0.5)			
Men	6	1.8±0.4*	10±1	7±2	0.9±0.2	75±6	90±8	1.2±0.1	3.7±0.7	<0.2	54±5	<19	<0.2
Premenopausal women	6	1.7±0.5	24±10	9±2	1.0±0.1	66±5	92±7	1.6±0.1	3.3±0.6	0.25±0.03**	45±5	17±4**	0.3±0.04**
Postmenopausal women	3	3.3±2.3	57±16	92±7	2.5±0.3	58±3	211±28	2.6±0.4	8.2±1.9	0.3±0‡‡	41±1	18±0.5‡‡	0.3±0.1
Primary hypothyroid													
Premenopausal women	4	89±31	19±6	23±11	2.5±0.6	60±8	202±26	5.9±1.3	4.8±1.3	0.6±0.1	40±4	33±8	2.6±1.0
Postmenopausal women	4	97±30	78±17	112±11	3.9±1.6	49±7	277±138¶	5.9±2.3	8.6±4.0	0.5±0.1	34±3	23±5	1.6±0.2
Central hypothyroid													
Woman with normal sella turcica	1	1.0	1.2	2.5	<0.5	43	<31	<0.5	<0.5	<0.2	—	—	<0.2
Woman with enlarged sella turcica	1	5.2	23§	33§	0.8§	61	71	2.0	—	<0.2	—	—	0.3
Hyperthyroid													
Premenopausal women	3	<0.5	12±5	12±5	0.7±0.1	71±4	66±10	0.7±0.2	2.2±0.4	<0.2	61±6	<19	<0.2
Postmenopausal women	2	<0.5	71±10	92±25	2.5±0.6	65±8	237±81	2.3±0.4	9.5±5.0	<0.2	58±8	<19	<0.2

* Values are presented as mean±SE.
‡ In postmenopausal women, normal hLH values were 40–95 mIU/ml and hFSH values, 35–210 mIU/ml.
§ This woman was postmenopausal.
‖ In postmenopausal women, normal alpha values were 1–7 ng/ml.
¶ Alpha secretion rates were 181, 550, and 98 μg/day/m^2; serum alpha concentrations were 3.4, 8.3, and 1.1 ng/ml, respectively.
** Two normal premenopausal women had undetectable hTSH-β concentrations (<0.2 ng/ml) basally and post-TRH.
‡‡ One normal postmenopausal woman had an undetectable hTSH-β concentration (<0.2 ng/ml) basally.

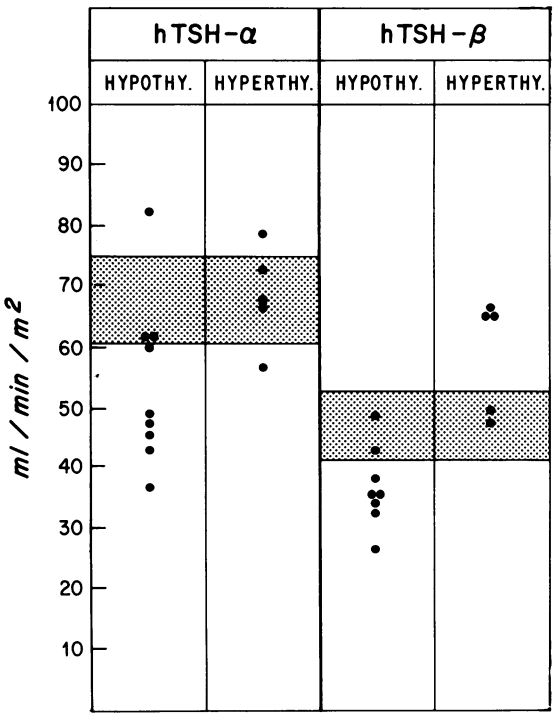

FIGURE 4 MCR (milliliters per minute per square meters) of hTSH-α and hTSH-β in hypothyroid and hyperthyroid patients. The shaded areas represent MCR of the normal individuals studied (mean±2SE). Mean MCR of both subunits were significantly slower in the hypothyroid patients than in the normals. MCR of subunits were not faster in the hyperthyroid patients than in normals.

MCR of 49±4 ml/min per m² (paired t test, $P < 0.005$). In addition, the mean MCR of hCG-α of 61±3 ml/min per m² was significantly slower ($P < 0.001$) than the simultaneously determined hTSH-α MCR. MCR of both hTSH-α and hCG-α were determined in the normal individuals since the structures of the alpha subunits of hTSH, hFSH, and hLH are thought to be virtually identical, whereas hCG-α has been suggested to have a higher carbohydrate content. The three normal postmenopausal women studied had MCR of subunits similar to those of the other normal subjects.

MCR of subunits were also determined in patients with disorders of thyroid function (Fig. 4 and Table I). MCR in nine hypothyroid patients (seven primary and two central) were significantly slower at 55±5 ml/min per m² for hTSH-α (unpaired t test, $P < 0.05$) than in normals. Likewise, MCR of hTSH-β in eight patients with primary hypothyroidism were significantly slower at 37±2 ml/min per m² ($P < 0.05$) than in normals. However, in the five hyperthyroid patients studied, MCR of hTSH-α (69±4 ml/min per m²) and hTSH-β (59±4 ml/min per m²) were not significantly different from those of the normal individuals. Similarly to normals, hTSH-α MCR were more rapid than those of hTSH-β in the hypothyroid and the hyperthyroid patients.

Correlation of MCR of subunits of hTSH with thyroid hormone concentrations and creatinine clearance. The correlation between serum thyroid hormone concentrations and MCR of both subunits of hTSH in the 15 normal, 10 hypothyroid, and 5 hyperthyroid subjects studied were investigated. T_4 was related to MCR of hTSH-α with a correlation coefficient (r) = 0.41 ($P < 0.05$), and to MCR of hTSH-β with r = 0.53 ($P < 0.005$) (Fig. 5). T_3 was related to MCR of hTSH-α with r = 0.32 ($P > 0.05$), and to MCR of hTSH-β with r = 0.54 ($P < 0.005$). Likewise, creatinine clearance of all the subjects was related to the MCR of hTSH-α with r = 0.46 ($P < 0.02$), and MCR of hTSH-β with r = 0.28 ($P > 0.10$) (Fig. 6). All of the subjects studied had normal serum creatinine concentrations, and none had known renal disease.

Secretion rates of subunits of hTSH. Fluctuations in serum alpha concentrations were small in six normal individuals (two men, two premenopausal women, and two postmenopausal women), who had serum alpha measured every 20 min throughout a day (Fig. 7). In addition, several individuals in this study had serum alpha measured every 30 min for 4½ h with similar results. These findings permitted calculation of daily secretion rates of free alpha subunits. The alpha secretion rates (mean±SE) in the six normal men and six normal premenopausal women studied were similar and averaged 91±5 μg/day per m² (Table I). The three normal postmenopausal women had an increased alpha secretion rate of 211±28 μg/day per m². The four premenopausal women with primary hypothyroidism had an alpha secretion rate of 202±26 μg/day per m²; the three postmenopausal hypothyroid women, 277 ±138 μg/day per m². The postmenopausal hypothyroid women had wide variations in their alpha secretion rates because of marked differences in their serum alpha concentrations rather than in their MCR of hTSH-α. However, the alpha secretion rate was significantly increased in the patients with primary hypothyroidism as compraed to the normals whether the postmenopausal individuals were included ($P < 0.02$) or excluded ($P < 0.001$). The three premenopausal hyperthyroid women had an alpha secretion rate of 66±10 μg/day per m²; the two postmenopausal hyperthyroid women, 237±81 μg/day per m². The alpha secretion rate was significantly decreased only when the three premenopausal hyperthyroid women were compared to the six normal men and six normal postmenopausal women ($P < 0.05$). In addition, the two patients with central hypothyroidism had decreased alpha secretion rates of <31 and 71 μg/day per m² (Table I). Alpha secretion rates in all the subjects studied were significantly related to the peak serum alpha after TRH (r = 0.84, $P < 0.001$) and after LHRH (r = 0.82, $P < 0.001$) (Table I).

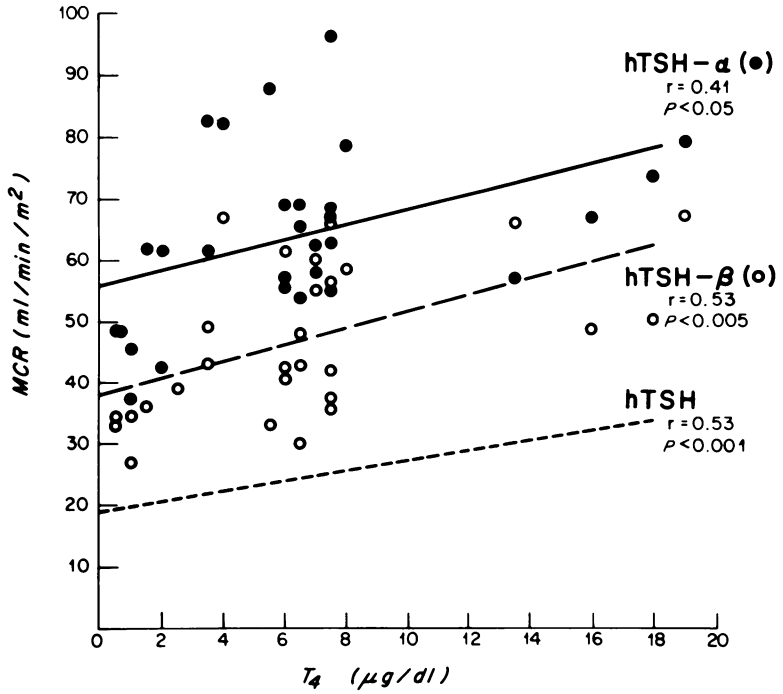

FIGURE 5 Correlation of serum T_4 with MCR (milliliters per minute per square meters) of hTSH-α (●) and of hTSH-β (○). The regression line for the correlation between T_4 and complete hTSH MCR is reproduced from our previously published data (11).

Endogenous serum hTSH-β was <0.2 ng/ml in all the hyperthyroid patients and in 9 of the 15 normal individuals studied, and was 0.3±0.02 ng/ml in the six normals with detectable concentrations; hTSH-β was elevated in the patients with primary hypothyroidism at 0.6±0.1 ng/ml (Table I). Significant increases in serum hTSH-β occurred after TRH only in the patients with primary hypothyroidism in spite of the greater sensitivity of this hTSH-β immunoassay. In the six normal women with detectable serum hTSH-β, the calculated secretion rate of hTSH-β was 17±2 μg/day per m^2; in the other normals and the hyperthyroid patients, hTSH-β secretion rate was <19 μg/day per m^2. The secretion rate of hTSH-β in the eight patients with primary hypothyroidism was significantly increased at 28±5 μg/day per m^2 when compared to that of normals ($P < 0.005$).[2]

DISCUSSION

The method of constant infusion to equilibrium for determination of MCR requires that a steady state of labeled subunit in serum has been achieved (Fig. 1) and that labeled and unlabeled subunits have similar clearance rates. It has previously been shown that labeled and unlabeled hTSH and hLH had similar MCR in humans (20, 21), and that labeled and unlabeled hTSH had similar MCR in dogs (22). Due to insufficient quantities of subunits available, studies with unlabeled subunits have not yet been performed. However, the method used for iodination of subunits in this study has previously been shown to preserve the biological activity of complete TSH (13). Moreover, labeled subunits had similar elution volumes to unlabeled preparations by gel chromatography, and only the monomeric labeled subunits were immunoreactive and thus included in the calculation of MCR (Fig. 2).

We have found MCR of hTSH-α and hTSH-β in normal individuals to be two to three times as rapid as that of hTSH (Fig. 3), which was 25 ml/min per m^2 (11), and as much as 5–30 times as rapid as that of the gonadotropins (23–25). The clearance of subunits of hLH has recently been reported by a single injection method to be twice as rapid as that of hLH (21). Although hCG-α and hTSH-α were simultaneously infused for determination of MCR in the normal individuals, hTSH-α had a more rapid MCR than hCG-α. The reason for the slower MCR of hCG-α may be either a greater carbohydrate and sialic acid content than the other alpha subunits (26) or artifactual differences in the particular two preparations

[2] For tests of significance, the normal individuals with undetectable serum hTSH-β were assigned a serum concentration equivalent to the detection limit of the assay. This serum concentration (0.2 ng/ml) was multiplied by the respective MCR of hTSH-β to yield a maximum hTSH-β secretion rate.

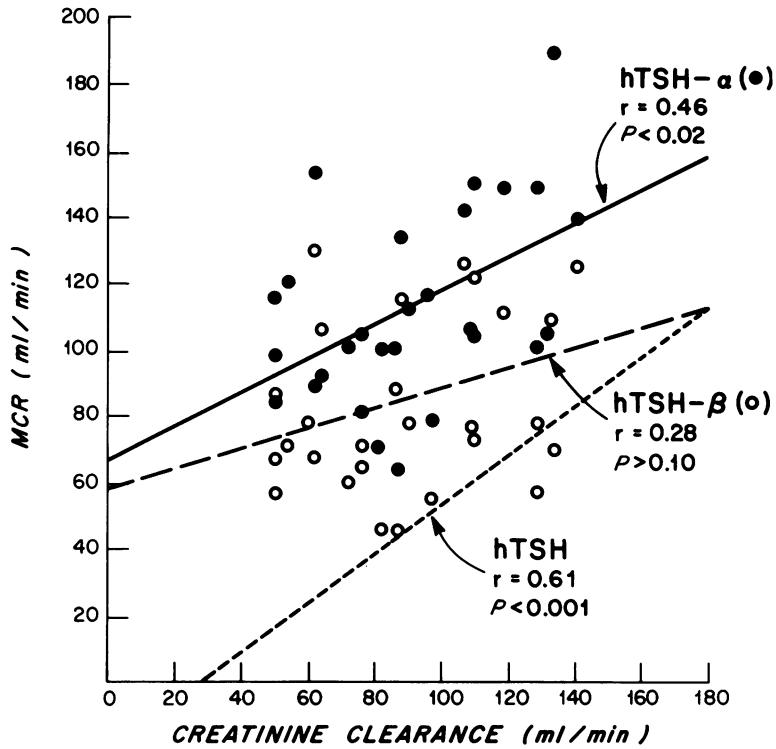

FIGURE 6 Correlation of creatinine clearance with MCR (milliliters per minute) of hTSH-α (●) and of hTSH-β (○). MCR is here expressed as milliliters per minute since creatinine clearance is dependent on body surface area. The regression line for the correlation between creatinine clearance and hTSH MCR is reproduced from our previously published data (11).

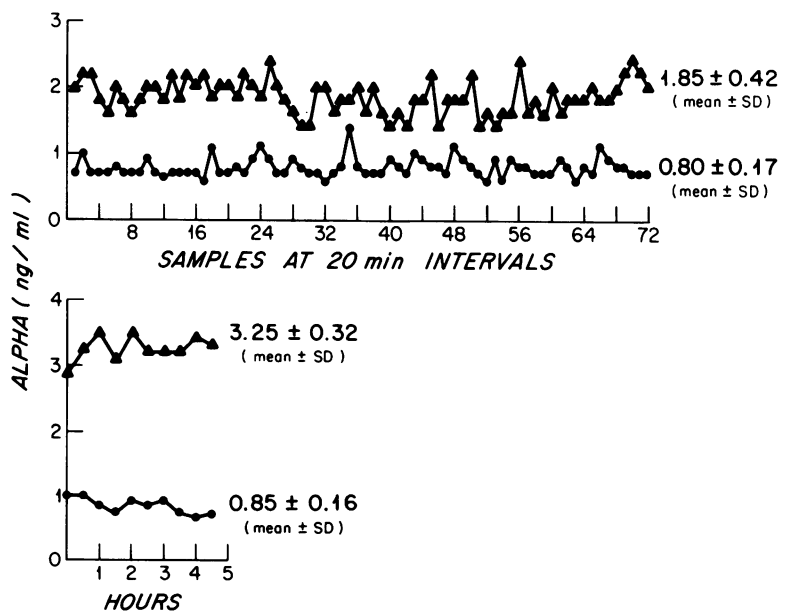

FIGURE 7 Serum alpha concentrations in a representative normal postmenopausal woman (▲) and a premenopausal woman (●) every 20 min for 1 day (upper panel). Serum alpha concentrations in representative study subjects, a postmenopausal hypothyroid woman (▲) and a normal premenopausal woman (●) every 30 min for 4½ h (lower panel). Alpha fluctuations were only slightly greater than could be accounted for by the 8% coefficient of variation in the alpha immunoassay.

of alpha subunits arising during their dissociation and purification from complete glycoprotein hormones. In addition, hTSH-α had a faster MCR than hTSH-β in all the individuals studied (Figs. 3, 4) even though both subunits have approximately the same molecular weight and hTSH-α has a greater carbohydrate content (27). It has been shown previously that in certain cases proteins with more carbohydrate, specifically sialic acid, are protected from degradation in the liver and have slower clearances (28). However, our data have shown that other factors must also be important in determining the clearance of glycoproteins.

Since MCR of subunits were influenced by body surface area (Table I and Fig. 3), all MCR in this report have been expressed as milliliters per minute per square meters. MCR of subunits of hTSH were significantly correlated with the serum T_4 levels of the subjects studied (Fig. 5), but not always with the serum T_3 levels. MCR of subunits in hypothyroid patients (Fig. 4) were significantly slower than those of normal individuals; furthermore, study of a larger group of hyperthyroid patients might have yielded significantly faster MCR of subunits than in normal individuals. Because MCR was also correlated with creatinine clearance (Fig. 6), excretion of subunits in urine was investigated but could not be adequately determined because of renal deiodination of the trace quantities of labeled subunits.

The lack of significant diurnal variation in serum alpha concentrations (Fig. 7), in spite of fluctuations throughout the day in serum gonadotropins (29, 30), allowed the calculation of daily secretion rates of alpha subunits. Since immunologically indistinguishable alpha subunits are secreted from both hTSH- and gonadotropin-secreting pituitary cells (2, 7), secretion rates of total alpha subunits from the pituitary were calculated (Table I). Secretion rate of subunits has been used instead of production rate since subunits produced in the pituitary can also be utilized to make complete glycoprotein hormones.

The patients with primary hypothyroidism had elevated daily alpha secretion rates in spite of lower MCR of hTSH-α, whereas the normal postmenopausal women had elevated alpha secretion rates with normal hTSH-α MCR. The premenopausal hyperthyroid women had significantly decreased alpha secretion rates in spite of MCR of hTSH-α not different from normals. The two patients with central hypothyroidism, who also had inappropriately low gonadotropins, had even smaller alpha secretion rates than the hyperthyroid patients, presumably because of decreased alpha secretion from both gonadotrophs and thyrotrophs (Table I). Therefore, as we had previously reported for hTSH (11), changes in serum alpha were due more to altered pituitary alpha secretion rather than to changes in MCR. Secretion rates of hTSH-β were also significantly elevated in patients with primary hypothyroidism. We have previously reported that there was a 50-fold increase in hTSH secretion rates in primary hypothyroidism (11); however, there was only a two-fold increase in alpha secretion rates in primary hypothyroidism (202 μg/day per m^2) as compared to normal (91 μg/day per m^2), as well as in hTSH-β secretion rates (28 vs. 17 μg/day per m^2). Thus, it appears that most of the increased synthesis of subunits of hTSH, particularly of the unique beta subunit, is utilized for production of hTSH. Nevertheless, in primary hypothyroidism acute secretion of subunits of hTSH after TRH (Table I) was more comparable in percent increments to that of complete hTSH (2, 5), suggesting different "pools" of hTSH and subunits involved in acute vs. chronic secretion.

These studies have implications for the mechanism of biosynthesis of the glycoprotein hormones. The alpha secretion rates were much greater than those of hTSH-β in all patients. Furthermore, the mean alpha secretion rate of 91 μg/day per m^2 in normal individuals was also greater than those of both hTSH (11) and hFSH (24) and comparable to that of hLH (23) when expressed in molar terms. The high secretion rates of free subunits have not previously been appreciated in measurements of serum concentrations because of the much more rapid MCR of subunits compared to complete glycoprotein hormones. Much higher alpha than hTSH-β secretion rates were consistent with the previous finding of a marked predominance in normal pituitary glands of alpha subunits relative to both hTSH-β (2) and hLH-β (31, 32). Furthermore, certain pituitary tumors have been shown to secrete large quantities of alpha subunit without hTSH-β (7). In addition, there have been previous reports of isolated production of either alpha or beta subunit (18, 33), as well as differential response of alpha and beta subunits of hTSH in response to thyroid hormone (2). These data, as well as our current findings, are consistent with the concept that alpha and beta subunits of the glycoprotein hormones are independently synthesized.

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REFERENCES

1. Kourides, I. A., B. D. Weintraub, M. A. Levko, and F. Maloof. 1974. Alpha and beta subunits of human thyrot-

ropin: Purification and development of specific radioimmunoassays. *Endocrinology.* **94:** 1411–1421.
2. Kourides, I. A., B. D. Weintraub, E. C. Ridgway, and F. Maloof. 1975. Pituitary secretion of free alpha and beta subunit of human thyrotropin in patients with thyroid disorders. *J. Clin. Endocrinol. Metab.* **40:** 872–885.
3. Vaitukaitis, J. L., G. T. Ross, L. E. Reichert, Jr., and D. N. Ward. 1972. Immunologic basis for within and between species cross-reactivity of luteinizing hormone. *Endocrinology.* **91:** 1337–1342.
4. Binoux, M., J. G. Pierce, and W. D. Odell. 1974. Radioimmunological characterization of human thyrotropin and its subunits: Applications for the measurement of human TSH. *J. Clin. Endocrinol. Metab.* **38:** 674–682.
5. Kourides, I. A., B. D. Weintraub, E. C. Ridgway, and F. Maloof. 1973. Increase in the beta subunit of human TSH in hypothyroid serum after thyrotropin releasing hormone. *J. Clin. Endocrinol. Metab.* **37:** 836–840.
6. Hagen, C., and A. S. McNeilly. 1975. The specificity and application of a radioimmunoassay for the α-subunit of luteinizing hormone in man. *Acta Endocrinol.* **78:** 664–674.
7. Kourides, I. A., B. D. Weintraub, S. W. Rosen, E. C. Ridgway, B. Kliman, and F. Maloof. 1976. Secretion of alpha subunit of glycoprotein hormones by pituitary adenomas. *J. Clin. Endocrinol. Metab.* **43:** 97–106.
8. Hagen, C., and A. S. McNeilly. 1975. Changes in circulating levels of LH, FSH, LHβ- and α-subunit after gonadotropin-releasing hormone, and of TSH, LHβ- and α-subunit after thyrotropin-releasing hormone. *J. Clin. Endocrinol. Metab.* **41:** 466–470.
9. Edmonds, M., M. Molitch, J. G. Pierce, and W. D. Odell. 1975. Secretion of alpha subunits of luteinizing hormone (LH) by the anterior pituitary. *J. Clin. Endocrinol. Metab.* **41:** 551–555.
10. Edmonds, M., M. Molitch, J. G. Pierce, and W. D. Odell. 1975. Secretion of alpha and beta subunits of TSH by the anterior pituitary. *Clin. Endocrinol.* **4:** 525–530.
11. Ridgway, E. C., B. D. Weintraub, and F. Maloof. 1974. Metabolic clearance and production rates of human thyrotropin. *J. Clin. Invest.* **53:** 895–903.
12. Kourides, I. A., R. N. Re, B. D. Weintraub, E. C. Ridgway, and F. Maloof. 1975. Metabolic clearance (MCR) and production rates (PR) of subunits of human thyrotropin (hTSH), *Endocrinology.* **96:** 155. (Abstr.)
13. Goldfine, I. D., S. M. Amir, A. W. Petersen, and S. H. Ingbar. 1974. Preparation of biologically active ^{125}I-TSH. *Endocrinology.* **95:** 1228–1233.
14. Greenwood, F. C., W. M. Hunter, and J. S. Glover. 1963. The preparation of ^{131}I-labelled growth hormone of high specific radioactivity. *Biochem. J.* **89:** 114–123.
15. Tait, J. F. 1963. Review: The use of isotopic steroids for the measurement of production rates *in vivo*. *J. Clin. Endocrinol. Metab.* **23:** 1285–1297.
16. Ridgway, E. C., B. D. Weintraub, J. L. Cevallos, M. C. Rack, and F. Maloof. 1973. Suppression of pituitary TSH secretion in the patient with a hyperfunctioning thyroid nodule. *J. Clin. Invest.* **52:** 2783–2792.
17. Rosen, S. W., and B. D. Weintraub. 1971. Monotropic increase of serum FSH correlated with low sperm count in young men with idiopathic oligospermia and aspermia. *J. Clin. Endocrinol. Metab.* **32:** 410–416.
18. Weintraub, B. D., and S. W. Rosen. 1973. Ectopic production of the isolated beta subunit of human chorionic gonadotropin. *J. Clin. Invest.* **52:** 3135–3142.
19. Gautvik, K. M., A. H. Tashjian, Jr., I. A. Kourides, B. D. Weintraub, C. T. Graeber, F. Maloof, K. Suzuki, and J. E. Zuckerman. 1974. Thyrotropin-releasing hormone is not the sole physiologic mediator of prolactin release during suckling. *N. Engl. J. Med.* **290:** 1162–1165.
20. Odell, W. D., R. D. Utiger, J. F. Wilber, and P. G. Condliffe. 1967. Estimation of the secretion rate of thyrotropin in man. *J. Clin. Invest.* **46:** 953–959.
21. Pepperell, R. J., D. M. de Kretser, and H. G. Burger. 1975. Studies on the metabolic clearance rate and production rate of human luteinizing hormone and on the initial half-time of its subunits in man. *J. Clin. Invest.* **56:** 118–126.
22. Ridgway, E. C., F. R. Singer, B. D. Weintraub, L. Lorenz, and F. Maloof. 1974. Metabolism of human thyrotropin in the dog. *Endocrinology.* **95:** 1181–1185.
23. Kohler, P. O., G. T. Ross, and W. D. Odell. 1968. Metabolic clearance and production rates of human luteinizing hormone in pre- and postmenopausal women. *J. Clin. Invest.* **47:** 38–47.
24. Coble, Y. D., Jr., P. O. Kohler, C. M. Cargille, and G. T. Ross. 1969. Production rates and metabolic clearance rates of human follicle-stimulating hormone in premenopausal and postmenopausal women. *J. Clin. Invest.* **48:** 359–363.
25. Wide, L., E. Johannisson, K. -G. Tillinger, and E. Diczfalusy. 1968. Metabolic clearance of human chorionic gonadotropin administered to nonpregnant women. *Acta Endocrinol.* **59:** 579–594.
26. Bellisario, R., R. B. Carlsen, and O. P. Bahl. 1973. Human chorionic gonadotropin. Linear amino acid sequence of the α-subunit. *J. Biol. Chem.* **248:** 6796–6809.
27. Cornell, J. S., and J. G. Pierce. 1973. The subunits of human pituitary thyroid stimulating hormone. Isolation, properties, and composition. *J. Biol. Chem.* **248:** 4327–4333.
28. Ashwell, G. 1973. The role of sialic acid in the immunological and biological properties of glycoprotein hormones. *In* Proceedings of the 4th International Congress of Endocrinology. R. O. Scow, editor. Excerpta Medica, Amsterdam. 601–604.
29. Boyar, R., M. Perlow, L. Hellman, S. Kapen, and E. Weitzman. 1972. Twenty-four hour pattern of luteinizing hormone secretion in normal men with sleep stage recording. *J. Clin. Endocrinol. Metab.* **35:** 73–81.
30. Naftolin, F., H. L. Judd, and S. S. C. Yen. 1973. Pulsatile patterns of gonadotropins and testosterone in man: The effects of clomiphene with and without testosterone. *J. Clin. Endocrinol. Metab.* **36:** 285–288.
31. Prentice, L. G., and R. J. Ryan. 1975. LH and its subunits in human pituitary, serum and urine. *J. Clin. Endocrinol. Metab.* **40:** 303–312.
32. Hagen, C., and A. S. McNeilly. 1975. Identification of human luteinizing hormone, follicle-stimulating hormone, luteinizing hormone β-subunit and gonadotrophin α-subunit in foetal and adult pituitary glands. *J. Endocrinol.* **67:** 49–57.
33. Rosen, S. W., and B. D. Weintraub. 1974. Ectopic production of the isolated alpha subunit of the glycoprotein hormones. A quantitative marker in certain cases of cancer. *N. Engl. J. Med.* **290:** 1441–1447.