Effects of Experimentally Induced Mild Hyperthyroidism on Growth Hormone and Insulin Secretion and Sex Steroid Levels in Healthy Young Men

Jennifer C. Lovejoy, Steven R. Smith, George A. Bray, Johannes D. Veldhuis, Jennifer C. Rood, and Richard Tulley

Although triiodothyronine (T_3) exerts major regulatory actions in both animals and humans, most clinical studies of T_3 administration have been relatively short-term. The present study examined the effects of more than 2 months (63 days) of low-dose T_3 treatment on overnight pulsatile growth hormone (GH) secretion, short-term insulin secretion, and of sex steroid levels in seven healthy, lean men studied at an inpatient metabolic unit. At baseline, there were strong correlations between sex hormone–binding globulin (SHBG) and several measures of GH production, including total GH production ($r = .99$), GH interburst interval ($r = -.75$), and GH mass ($r = .82$). SHBG was also inversely correlated with basal insulin secretion ($r = -.74$). There was a 42% increase in serum levels of total testosterone (18.5 ± 1.3 to 26.3 ± 1.8 nmol/L, $P = .005$) and a 150% increase in SHBG (18.0 ± 2.2 to 44.9 ± 7.0 nmol/L, $P = .008$) following T_3 treatment. Estradiol and free testosterone levels were unchanged by treatment, although free testosterone decreased from 142.8 ± 18.4 to 137.3 ± 19.5 pmol/L. T_3 treatment significantly reduced the GH interburst interval ($P < .05$) and produced slight increases in the measures of GH secretion. There were no statistically significant effects of T_3 treatment on insulin secretion, although insulin peak amplitude, mass secreted per burst, and total production all decreased. We conclude that experimentally induced T_3 excess in healthy men produces significant and sustained changes in sex hormone levels and GH secretion. Furthermore, there are strong associations between SHBG and both GH and insulin secretion independent of thyroid hormone excess that require additional study.

SEVERAL LINES OF EVIDENCE suggest that hyperthyroidism results in abnormalities in other hormonal systems. Data from the early 1970s suggested that hyperthyroid patients exhibit alterations in sex steroid and gonadotropin levels.[1-4] More recently, growth hormone (GH) pulsatile secretion has been shown to be altered in hyperthyroid patients.[5] Furthermore, in animal studies, treatments that decrease thyroid function have been associated with increased insulin secretion and decreased insulin action.[6] Thus, the effects of hyperthyroidism on the function of other endocrine systems appear to be wide-ranging.

As a preliminary part of a major study to investigate the usefulness of low-dose thyroid hormone treatment as an addition to the standard ground-based model of simulated space flight (bedrest with a 6° head-down tilt), we administered triiodothyronine (T_3) to ambulatory, healthy volunteers for 63 days. During this period of experimentally induced mild hyperthyroidism, pulsatile GH and insulin secretion were assessed and the serum was analyzed for androgen and estrogen levels. Additionally, we examined the relationships between sex steroid and sex hormone–binding globulin (SHBG) levels and pulsatile hormone secretion prior to administration of thyroid hormone.

The purpose of this study was to characterize the effects of 9 weeks' treatment with low-dose T_3 on hormone (GH and insulin) secretion and sex hormone levels in healthy young men participating in research designed to improve on the ground-based model of spaceflight. We hypothesized that experimentally induced mild hyperthyroidism would increase GH secretion, as well as sex steroid levels. Furthermore, since thyroid hormones have been suggested to modulate insulin sensitivity, we hypothesized that T_3 treatment might reduce insulin secretion.

SUBJECTS AND METHODS

Subjects

Seven men were studied for 77 days during this inpatient study (for details see Lovejoy et al[7]). The subjects were 26.0 ± 2.2 years old (mean ± SE) and had an initial body mass index of 22.9 ± 1.4 kg/m^2. All subjects were healthy by laboratory and clinical measures. Written informed consent was obtained from each subject, and the protocol was approved by the Louisiana State University Institutional Review Board.

Experimental Design

The experimental design has been described previously.[7] Briefly, all individuals lived at the Metabolic Research Unit for the duration of the protocol but were permitted to leave for work or school during the day. Physical activity was controlled, and subjects were randomly assigned to either a high-fat (50% fat and 35% carbohydrate) or low-fat (20% fat and 65% carbohydrate) diet. Since the diet did not significantly influence any of the variables assessed in the present report, the data have been collapsed across dietary treatments. Although sexual activity (which might influence hormone levels) was not specifically controlled, visitors to the Metabolic Unit were limited and all visits were monitored with the subjects' knowledge by infrared cameras located in the subjects' rooms. T_3 treatment was started following a 3-week run-in period and continued for 9 weeks. All subjects initially received T_3 75 μg/d (liothyronine sodium; Cytomel; Smith-Kline-Beecham, Philadelphia, PA) in five divided doses every 4 hours during waking hours beginning at 6:00 AM, as previously described.[7] This dose was reduced if a subject became symptomatic or if serum T_3 levels were 2 SD above the mean for the T_3 assay in our laboratory (~4.6 nmol/L). All but one subject were dose-reduced to either 62.5 or 50 μg/d T_3 administered following the same daily schedule.

From the Pennington Biomedical Research Center and Louisiana State University School of Medicine, Baton Rouge, LA; and the University of Virginia Health Sciences Center and National Science Foundation Center for Biological Timing, Charlottesville, VA.

Submitted December 10, 1996; accepted June 25, 1997.

Supported by a grant from the National Aeronautics and Space Administration (NAG 9-714).

Address reprint requests to Jennifer C. Lovejoy, PhD, Pennington Biomedical Research Center, 6400 Perkins Rd, Baton Rouge, LA 70808-4124.

0026-0495/97/4612-0009$03.00/0

Endocrine Measures

Sampling for hormone secretion was performed at baseline and during week 9 of T_3 treatment. Episodic secretion of GH was measured using a waveform-specific deconvolution technique[8,9] applied to serum GH samples (3 mL) collected every 10 minutes from 9:00 PM to 9:00 AM. This technique defines all measured plasma GH concentrations in relation to the (1) number, (2) amplitude, and (3) duration of significant GH secretory bursts, as well as the subject-specific hormone half-life. Additionally, a novel approximate entropy (ApEn) statistic was used to evaluate the relative degree of serial orderliness of the GH concentration profiles. A larger ApEn value reflects greater apparent randomness (or less consistent repetition of subpatterns).[10]

Short-term insulin pulsatility was determined as described by Peiris et al.[11] After an overnight fast, an intravenous cannula was inserted into the antecubital vein. A heating pad was used to obtain arterialized venous samples. Beginning at 7:30 AM, blood samples (3 mL) were withdrawn every 2 minutes for 90 minutes for determination of peripheral insulin concentration. Deconvolution analysis was used to determine the frequency amplitude, pulse area, interpulse interval, and short-term insulin oscillatory peak using a previously validated model.[12]

Testosterone (total and free), estradiol, and SHBG were determined in single fasting serum samples collected between 7:00 and 8:00 AM in both pretreatment and posttreatment conditions.

Analytical Methods

The serum GH level was measured using an ultrasensitive chemiluminescent assay (Nichols, San Juan Capistrano, CA) with a sensitivity of 0.02 ng/mL and a within-assay coefficient of variation of 4% to 5% in these studies. Thyroid hormone levels were measured on an IMx analyzer (Abbott, Abbott Park, IL) using either a microparticle enzyme immunoassay (T_3 and thyrotropin [TSH]) or a fluorescence polarization immunoassay (thyroxine [T_4]). Testosterone, free testosterone, and SHBG levels were measured by radioimmunoassay using commercial kits (DPC, Los Angeles, CA). Estradiol was assayed on an automated IMx analyzer (Abbott).

Statistical Analysis

Data were analyzed using SAS for Windows (SAS Institute, Cary, NC). Standard univariate statistics were calculated to determine the means and variances and to assess the normality of the data. All data are reported as the mean ± SEM unless otherwise indicated. Changes over time with treatment for measures performed at baseline and 9 weeks were performed by calculating the difference from baseline for each subject and assessing whether the delta values were significantly different from zero using a paired, two-tailed Student's *t* test. Nonparametric statistics (Wilcoxon sign–rank tests) were used to analyze GH secretory data. Spearman nonparametric correlations were used to assess the relationship between endocrine variables at baseline, before T_3 treatment. An α level less than .05 was considered significant.

RESULTS

As we reported previously,[7] T_3 levels reached a plateau at 3.3 ± 0.3 nmol/L after 1 month of treatment (an increase of ~200% above baseline) and remained at this level for the duration of the study. Serum T_4 decreased by greater than 50%, on average, to reach a nadir of 36.5 ± 9.6 nmol/L, and TSH was suppressed to undetectable levels (<0.03 mU/L) in all subjects throughout the treatment period.[7]

We examined the relationships among various hormonal variables in these subjects at baseline, before initiating T_3 treatment. There were significant correlations between sex steroids and several measures of hormone pulsatility (Table 1).

Table 1. Spearman Correlations (*r*) Between Hormone Levels and GH Secretion Parameters in Seven Healthy Men Before T_3 Treatment

	GH Secretion Parameter				
Hormone	Production	Half-life	Interval	Mass	Bursts
Estradiol	−.29	.86†	.21	−.18	.11
Testosterone	.21	.39	−.07	.50	−.44
Free testosterone	−.71*	.25	.79†	−.61	−.33
SHBG	.99‡	−.11	−.75*	.82†	.37

*$P < .10$.
†$P < .05$.
‡$P < .001$.

GH production and interburst interval were highly correlated with both free testosterone and SHBG, and GH mass secreted per burst was significantly correlated with SHBG. The relationship between GH production rate and SHBG at baseline was particularly striking ($r = .99$; Fig 1). SHBG was also significantly and inversely correlated with insulin basal secretion ($r = -.74$, $P = .05$; Fig 2) and showed a strong trend toward correlation with 24-hour integrated insulin production ($r = -.72$, $P = .06$). In contrast to a previous report,[13] SHBG did not correlate with the insulin interburst interval ($r = .26$).

Serum levels of testosterone and SHBG were significantly increased after T_3 treatment (Fig 3). Estradiol, in contrast, was unchanged. Free testosterone levels decreased slightly, suggesting that changes in total androgen levels were primarily due to changes in the binding protein.

Data for GH and insulin secretion are shown in Table 2. The GH interburst interval was significantly decreased after 9 weeks of T_3 treatment. There was also a decrease in the GH ApEn statistic that was of borderline statistical significance ($P = .06$). The GH half-life declined in five of seven men, and the number of detectable GH secretory bursts and the mass secreted per burst increased with T_3 treatment, although these differences were not significant. Basal insulin secretion increased slightly,

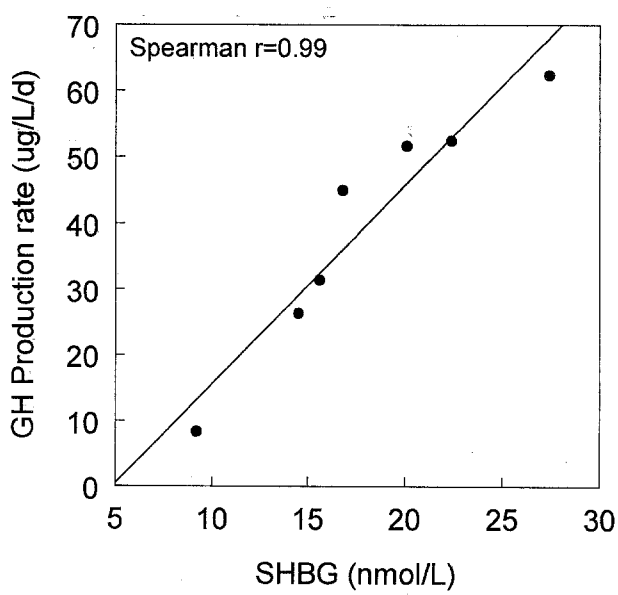

Fig 1. Correlation between SHBG and GH production in 7 healthy men pretreatment.

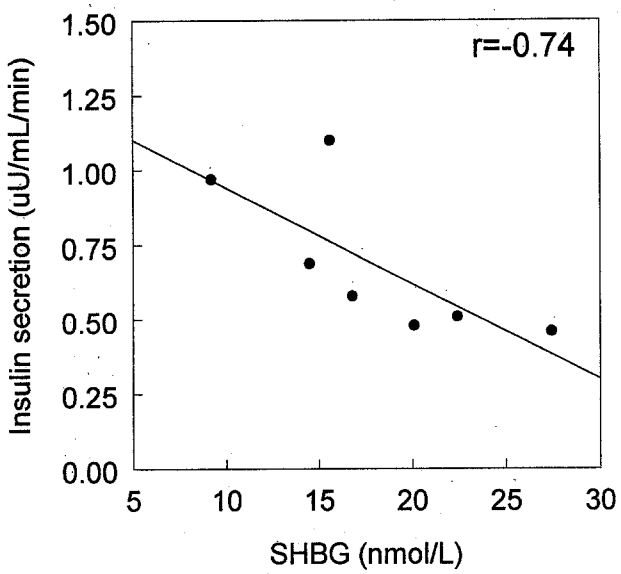

Fig 2. Correlation between SHBG and basal insulin secretion in 7 healthy men pretreatment.

whereas total insulin production, peak amplitude, and mass per secretion burst tended to decrease (not significant).

DISCUSSION

The present study investigated changes in the patterns of hormone secretion or hormone levels in response to treatment with relatively long-term, low-dose excess T_3 in healthy, lean men. We observed striking and novel correlations between SHBG and several measures of GH secretion, as well as correlations between SHBG and insulin secretion at baseline, before T_3 administration. T_3 treatment that induced mild hyperthyroidism resulted in significant changes in total testosterone levels and sex-steroid binding that persisted for 9 weeks. Furthermore, T_3 treatment induced persistent, albeit relatively small, changes in measures of GH and insulin secretion. These studies are unique in examining endocrine changes resulting from controlled and isolated T_3 excess, in contrast to those occurring in clinical conditions of acquired hyperthyroidism that are accompanied by longer-term and larger changes in body composition and may be confounded by concomitant pathophysiology (eg, Graves' disease).

Table 2. Measures of GH and Insulin Secretion Before and After T_3 Treatment for 9 Weeks in Seven Healthy Young Men (mean ± SEM)

Measure	Baseline	Post-T_3	*P*
GH			
Basal secretion ($\times 10^{-3}$ pg/L/min)	3.5 ± 1.4	5.9 ± 2.0	NS
Half-life (min)	17 ± 1.2	15 ± 1.5	NS
Mass (μg/L)	4.3 ± 0.8	5.3 ± 0.9	NS
Production rate (μg/L/d)	40 ± 7.0	50 ± 13	NS
No. of bursts	9.4 ± 0.9	11.6 ± 1.1	NS
Interburst interval (min)	72 ± 6.9	61 ± 5.5	.047
ApEn	0.6 ± 0.1	0.5 ± 0.1	.06
Insulin			
Basal secretion (μU/mL/min)	0.7 ± 0.1	0.8 ± 0.2	NS
No. of bursts	8.6 ± 0.9	8.4 ± 0.5	NS
Mass per burst (μU/mL)	4.8 ± 0.8	3.7 ± 0.7	NS
Peak amplitude (μU/mL/min)	1.2 ± 0.4	0.7 ± 0.1	NS
Production (μU/mL/90 min)	43.2 ± 9.8	31.7 ± 7.0	NS
Absolute increment (μU/mL)	2.3 ± 0.6	1.6 ± 0.3	NS

Previous studies from the 1970s have shown alterations in sex steroid levels in hyperthyroid males. Chopra et al[1-2] performed a series of studies in men with Graves' disease and reported that serum estradiol, total testosterone, and SHBG were increased. In the present study on men with experimental T_3 excess, we confirmed a significant increase in total testosterone and SHBG, but not estradiol. Free testosterone was measured in only one previous study of hyperthyroid patients,[2] to our knowledge, and was reported to be unchanged. Similarly, we did not observe a significant effect of T_3 treatment on free testosterone in the present study, supporting the suggestion of Chopra and Tulchinsky[2] that elevated testosterone levels in hyperthyroid patients are primarily due to increased bound testosterone occurring with the increase in SHBG. The lack of significant change in estradiol levels may reflect alterations in estrogen clearance, which we did not measure.

The present study also demonstrated several novel relation-

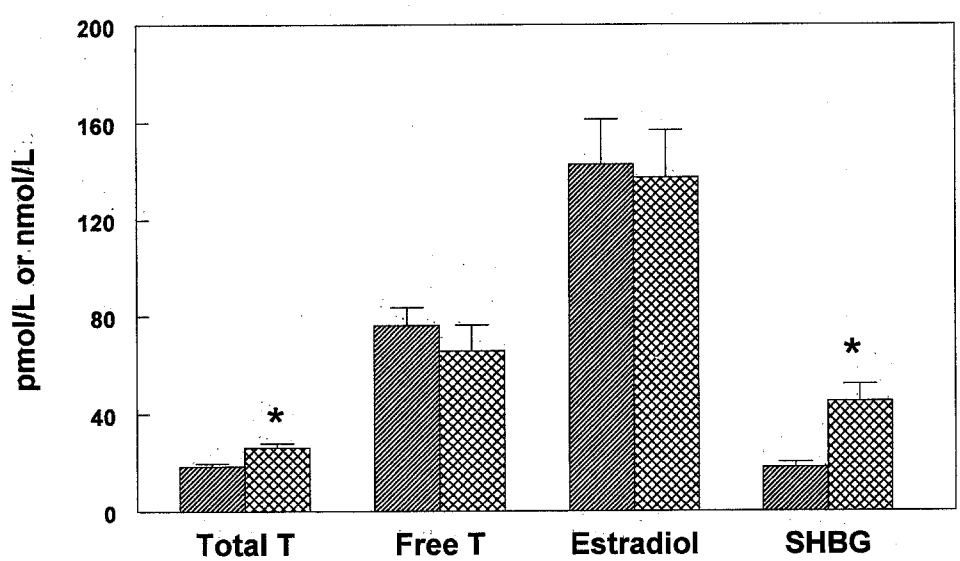

Fig 3. Changes in total and free testosterone, estradiol, and SHBG before (▨) and after (▩) 9 weeks of T_3 treatment in 7 healthy young men.

ships between measures of GH secretion, sex steroids, and SHBG in the subjects before T_3 administration. Several previous studies have demonstrated correlations between GH secretion and estradiol or testosterone levels[10,14,15]; however, SHBG has not been measured in these reports. We observed a striking positive correlation between GH production and SHBG ($r = .99$) and also found that the GH interburst interval and GH burst mass correlated with SHBG. SHBG has been previously reported to correlate positively with the GH half-life[16]; however, in contrast to the positive association between GH secretion and SHBG observed in the present study, administration of GH to GH-deficient patients decreases SHBG levels.[17] Understanding the relationship between GH secretion and SHBG will require additional, experimental studies. In this regard, it would have been helpful to have measurements of serum levels of insulin-like growth factor-1 (IGF-1) and its binding proteins. Unfortunately, in the present study, there was inadequate serum available to perform these analyses.

In addition to the relationships with SHBG, we also observed that free testosterone was significantly and inversely correlated with GH production and positively correlated with the GH interburst interval. Finally, we observed a strong correlation between estradiol levels and GH half-life ($r = .85$), which has been previously observed.[10] These data showing that GH secretion (both basal and pulsatile) is correlated with sex steroid levels support the hypothesis that sex steroids may, in part, control GH secretion.

SHBG has also been suggested to be an independent predictor of non–insulin-dependent diabetes mellitus,[18] and the concentration of SHBG appears to be regulated by insulin both in vitro and in vivo.[19,20] Peiris et al[13] have previously reported a strong correlation between insulin secretory pulse intervals in healthy men and SHBG levels ($r = .86$). We did not observe this relationship in the present study ($r = .26$); however, we did find SHBG to be significantly and inversely correlated with basal insulin secretion and 24-hour integrated insulin production. Previous studies have reported a similar inverse relationship between fasting insulin and SHBG.[21] It is thus unclear whether pulsatile insulin secretion or merely basal insulin secretion is the important regulator of SHBG. Future studies should address this question using deconvolution methodology and examining different populations.

Previous studies have suggested that patients with hyperthyroidism have altered GH secretion. Iranmanesh et al[5] reported that hyperthyroid men have a higher frequency of detectable GH secretory bursts and a greater mass of GH secreted per burst, resulting in an increase in the 24-hour production rate of GH of nearly fourfold. The GH interburst interval was significantly reduced in the present study, and there was a trend toward an increase in GH measured secretion and production rates, consistent with the previous report but reflecting the effects of a relatively low elevation in thyroid hormones induced by our treatment. Statistical power analysis indicated that the power to detect differences at the observed levels with seven subjects ranged from about 25% for GH production to about 88% for the interburst interval, with the power for the majority of GH secretion variables being 50% to 70%.

GH ApEn tended to decrease ($P = .06$) with T_3 treatment. ApEn is a recently validated statistic that quantifies the regularity or orderliness of a hormone-release profile over time.[22] ApEn is a barometer of feedback control within an axis, and monitors the degree of repetition of subpatterns across the data. It should be distinguished from classic Kolmogorov-Siani entropy and other measures of deterministic chaos, since it can be applied to short data series with as few as 50 observations.[23] In recent endocrine investigations, ApEn has been shown to increase with age for the GH axis[10] and the LH and testosterone axis in men.[24] In addition, obesity and the serum testosterone concentration correlate with more disorderly patterns of GH release in healthy men.[10] Consequently, ApEn offers a sensitive marker of the subtle, subordinate (ie, nonpulsatile) aspects of a hormone time series. Here, we identify for the first time an effect of isolated T_3 excess on the ApEn of GH release, thus suggesting that thyroid hormones control feedback coordination within the GH-IGF-1 axis. Further studies of GH and IGF-1 autofeedback and somatostatin and GHRH individual and concerted regulation of pituitary GH secretion during isolated T_3 excess will be required to explicate the particular mechanisms of altered rhythmic GH secretion that we observed here in response to T_3 action.

There were slight but nonsignificant decreases in insulin secretion measures with 9 weeks of T_3 treatment (Table 2). A previous study in sheep treated with proplylthiouracyl to decrease thyroid function showed that insulin secretion was enhanced while insulin action was decreased.[6] In rats, experimental hyperthyroidism increases insulin-stimulated glucose transport by increasing GLUT4 glucose transporters.[25] Similarly, in humans, glucose uptake has been reported to be increased when thyroid hormones are elevated,[26] even when insulin secretion is blocked by somatostatin.[27] Although we did not directly measure whole-body insulin sensitivity in the present study, our data are consistent with an increase in insulin sensitivity in subjects treated with low-dose thyroid hormone.

The present data thus support a theoretical model in which thyroid hormones, presumably acting through a central mechanism, result in increased activation of the GH axis and decreased insulin secretion (either through direct action or indirectly via changes in insulin sensitivity). These changes in GH and/or insulin may modulate the increases in SHBG, as well as the reported changes in androgen and estrogen levels in hyperthyroidism. Although thyroid hormones per se are known to regulate SHBG (as reviewed by Selby[28]), the relationships we observed between SHBG and GH and insulin were presumably independent of thyroid hormone stimulation, since they were observed before administration of T_3. Further testing of this potential unifying hypothesis is needed to fully delineate the pathways involved.

In summary, the present model of experimental hyperthyroidism indicates that controlled T_3 excess results in persistent changes in several measures of GH and insulin pulsatile secretion. Additionally, total testosterone and SHBG levels are significantly altered even with this low-dose thyroid hormone treatment. It should be noted that mild biochemical hyperthyroidism in women may or may not resemble what we observed here in men, since recent studies show that gender strongly modifies the effects of age, obesity, and physical fitness on the GH axis.[29] Thus, it will be important to perform separate studies in women. Finally, although correlation analyses cannot address causality, these data provide indirect support for two hypotheses: (1) sex

steroids are important regulators of GH secretion, and (2) insulin secretion is an important regulator of SHBG.

ACKNOWLEDGMENT

The authors would like to thank the volunteers who donated time and energy to this project. We are grateful to the nursing and clinical staff of the Pennington Biomedical Research Center, particularly Ricky Brock, RN, the research coordinator for the study. We also appreciate the excellent technical support of Mark Klemperer, MS, and Helena Duplantis, MS, RD. Finally, we are grateful for the helpful insights of our external Advisory Board for this project, consisting of Drs D. Clemmons, H. Lane, J. Nicoloff, and F. Svec.

REFERENCES

1. Chopra IJ, Abraham GE, Chopra U, et al: Alterations in circulating estradiol-17β in male patients with Graves' disease. N Engl J Med 286:124-129, 1972

2. Chopra IJ, Tulchinsky D: Status of estrogen-androgen balance in hyperthyroid men with Graves' disease. J Clin Endocrinol Metab 38:269-277, 1974

3. Southren AL, Olivo J, Gordon GG, et al: The conversion of androgens to estrogens in hyperthyroidism. J Clin Endocrinol Metab 38:207-214, 1974

4. Ruder H, Corval P, Mahoudeau JA: Effects of induced hyperthyroidism on steroid metabolism in man. J Clin Endocrinol Metab 33:382-387, 1971

5. Iranmanesh A, Lizarralde G, Johnson ML, et al: Nature of altered growth hormone secretion in hyperthyroidism. J Clin Endocrinol Metab 72:108-115, 1991

6. Achmadi J, Terashima Y: The effect of propylthiouracyl-induced low thyroid function on secretion response and action of insulin in sheep. Domest Anim Endocrinol 12:157-166, 1995

7. Lovejoy JC, Smith SR, Bray GA, et al: A paradigm of experimentally induced mild hyperthyroidism: Effects on nitrogen balance, body composition, and energy expenditure in healthy young men. J Clin Endocrinol Metab 82:765-770, 1997

8. Veldhuis JD, Johnson ML: Deconvolution analysis of hormone data. Methods Enzymol 210:539-575, 1992

9. Iranmanesh A, Lizarralde G, Veldhuis JD: Age and relative adiposity are specific negative determinants of the frequency and amplitude of growth hormone (GH) secretory bursts and the half-life of endogenous GH in healthy men. J Clin Endocrinol Metab 73:1081-1088, 1991

10. Veldhuis JD, Liem AY, South S, et al: Differential impact of age, sex steroid hormones, and obesity on basal versus pulsatile growth hormone secretion in men as assessed in an ultrasensitive chemiluminescence assay. J Clin Endocrinol Metab 80:3209-3222, 1995

11. Peiris AN, Stagner JI, Vogel RL, et al: Body fat distribution and peripheral insulin sensitivity in healthy men: Role of insulin pulsatility. J Clin Endocrinol Metab 75:290-294, 1992

12. Porksen N, Munn S, Steers J, et al: Pulsatile insulin secretion accounts for 70% of total insulin secretion during fasting. Am J Physiol 269:E478-E488, 1995

13. Peiris AN, Stagner JI, Plymate SR, et al: Relationship of insulin secretory pulses to sex-hormone binding globulin in normal men. J Clin Endocrinol Metab 76:279-282, 1993

14. Ho KY, Evans WS, Blizard RM, et al: Effects of sex and age on the 24-hour profile of growth hormone secretion in man: Importance of endogenous estradiol concentrations. J Clin Endocrinol Metab 64:51-58, 1987

15. Corpas E, Harman SM, Pineyro MA, et al: Growth hormone (GH)-releasing hormone-(1-29) twice daily reverses the decreased GH and insulin-like growth factor-I levels in old men. J Clin Endocrinol Metab 75:530-535, 1992

16. Holl RW, Schwarz U, Schauwecker P, et al: Diurnal variation in the elimination rate of human growth hormone (GH): The half-life of serum GH is prolonged in the evening, and affected by the source of the hormone, as well as by body size and estradiol. J Clin Endocrinol Metab 77:216-220, 1993

17. Gafny M, Silbergeld A, Klinger B, et al: Comparative effects of GH, IGF-1, and insulin on serum sex-hormone binding globulin. Clin Endocrinol (oxf) 41:169-175, 1994

18. Lindstedt G, Lundberg PA, Lapidus L, et al: Low sex-hormone binding globulin concentration as an independent risk factor for development of NIDDM: 12-yr follow-up of a population study of women in Gothenburg, Sweden. Diabetes 40:123-128, 1991

19. Plymate SR, Jones RE, Matej LA, et al: Regulation of sex-hormone binding globulin (SHBG) production in Hep G2 cells by insulin and prolactin. J Clin Endocrinol Metab 67:460-464, 1988

20. Nestler JE, Powers LP, Matt DW, et al: A direct effect of hyperinsulinemia on serum sex-hormone binding globulin levels in obese women with the polycystic ovary syndrome. J Clin Endocrinol Metab 72:83-89, 1991

21. Preziosi P, Barrett-Connor E, Papoz L, et al: Interrelation between plasma sex hormone–binding globulin and plasma insulin in healthy adult women: The Telecom Study. J Clin Endocrinol Metab 76:283-287, 1993

22. Pincus SM, Singer BH: Randomness and degrees of irregularity. Proc Natl Acad Sci USA 93:2083-2088, 1996

23. Pincus SM: Approximate entropy (ApEn) as a complexity measure. Chaos 5:110-117, 1995

24. Pincus SM, Mulligan T, Iranmanesh A, et al: Older males secrete luteinizing hormone and testosterone more irregularly, and jointly more asynchronously, than younger males: Dual novel facets. Proc Natl Acad Sci USA 93:14100-14105, 1996

25. Matthaei S, Trost B, Hamann A, et al: Effect of in vivo thyroid hormone status on insulin signalling and GLUT1 and GLUT4 glucose transport systems in rat adipocytes. J Endocrinol 144:347-357, 1995

26. Sandler MP, Robinson RP, Rabin D, et al: The effect of thyroid hormones on gluconeogenesis and forearm metabolism in man. J Clin Endocrinol Metab 56:479-485, 1983

27. Muller MJ, Burger AJ, Ferrannini E, et al: Glucoregulatory function of thyroid hormones: Role of pancreatic hormones. Am J Physiol 256:E101-E110, 1989

28. Selby C: Sex hormone binding globulin: Origin, function, and clinical significance. Ann Clin Biochem 27:532-541, 1990

29. Weltman A, Weltman JY, Hartman ML, et al: Relationship between age, percentage body fat, fitness and 24 hr growth hormone release in healthy young adults: Effects of gender. J Clin Endocrinol Metab 78:543-548, 1994