Acute and Prolonged Effects of Insulin-Induced Hypoglycemia on the Pituitary-Thyroid Axis in Humans

Bernd Schultes, Kerstin M. Oltmanns, Werner Kern, Jan Born, Horst L. Fehm, and Achim Peters

Secretory activity of the pituitary-thyroid axis and thyroid hormone metabolism show characteristic changes in response to different stressors often referred to as the euthyroid sick syndrome. Hypoglycemia is an acute metabolic stressor inducing various neuroendocrine responses, the effects of which on pituitary-thyroid secretory activity so far have been entirely neglected. We performed stepwise hypoglycemic and euglycemic clamps each lasting 6 hours in 30 healthy men. To assess the potential influence of hyperinsulinemia on pituitary-thyroid hormone release, 2 different rates of insulin infusion were used for the clamps. During the hypoglycemic clamps, serum thyroid-stimulating hormone (TSH) concentration decreased in comparison to the euglycemic condition on average by 28% ± 4% ($P < .001$), while serum concentration of free triiodothyronine (fT_3), free thyroxine (fT_4), and thyroxine-binding globulin (TBG) remained unchanged. The effect did not depend on the rate of insulin infusion. To assess the prolonged effect of acute hypoglycemia on pituitary-thyroid secretory activity, serum TSH and thyroid hormone concentrations were subsequently measured in another 15 healthy men before and 18 hours after 2 consecutive hypoglycemic clamps together lasting about 270 minutes. Compared with values before the hypoglycemic clamps, serum levels of TSH, fT_3, and fT_4 were found to be still reduced (by 44% ± 6%, 12% ± 2%, and 10% ± 1%, respectively) 18 hours after the last hypoglycemic episode ($P < .001$ for all comparisons). The observed hormonal changes after hypoglycemia were not accompanied by any change in resting energy expenditure (REE). Data indicate acute as well as prolonged inhibitory influences of hypoglycemia on pituitary-thyroid secretory activity. The pattern of changes suggests that hypoglycemia exerts its influence primarily at a central, ie, pituitary and/or hypothalamic, site of the axis.

SECRETORY ACTIVITY OF the pituitary-thyroid axis and thyroid hormone metabolism are well-known to respond with specific changes to different stressors, such as severe illness, surgery, or starvation. The pattern of this response has been often referred to as the euthyroid sick syndrome, nonthyroidal illness syndrome, or low triiodothyronine (T_3) syndrome.[1-3] First, a decrease in serum T_3 concentration occurs as a result of reduced peripheral conversion of thyroxine (T_4) to T_3. If the stressor continues, this response is followed by a decrease in serum thyroid-stimulating hormone (TSH) concentration and, later on, by a consecutive decrease in free (f)T_4.[4-6]

Hypoglycemia is an acute metabolic stressor inducing a profound and complex pattern of neuroendocrine responses, including a stimulation of the sympathoadrenal system and the hypothalamus-pituitary-adrenal (HPA) axis,[7] as well as a suppression of the gonadotropic axis.[8] Whether hypoglycemia also induces alterations in secretory activity of the pituitary-thyroid axis and thyroid hormone metabolism is so far unknown. To address this question, we measured serum concentrations of TSH, fT_4, fT_3, and TBG during stepwise hypoglycemic and euglycemic clamp experiments performed in young, healthy men. Experimental induction of hypoglycemia is accompanied necessarily by hyperinsulinemia, which per se is known to modulate secretory activity of several neuroendocrine axis.[9,10] Moreover, insulin has been shown to dose-dependently amplify neuroendocrine counterregulatory responses to hypoglycemia.[11,12] To assess whether the response of the pituitary-thyroid axis to hypoglycemia is likewise insulin dependent, clamp experiments were performed by using 2 different rates of insulin infusion. Because data analysis of this experiment showed a sole acute inhibitory effect of hypoglycemia on pituitary TSH release, we subsequently performed a second experiment to assess prolonged effects of hypoglycemia on the pituitary-thyroid axis, which could be of clinical relevance. Thyroid hormone concentrations were measured before, as well as 18 hours after 2 consecutive hypoglycemic clamps. Additionally, to assess whether hormonal changes are accompanied by changes in resting energy expenditure (REE), indirect calorimetry was performed before and 18 hours after the hypoglycemic clamps.

SUBJECTS AND METHODS

Subjects

Forty-five young, healthy men participated in the experiments, 30 in experiment 1 and 15 in experiment 2. Exclusion criteria were chronic or acute illness, current medication of any kind, smoking, alcohol or drug abuse, adiposity (body mass index [BMI] > 27 kg/m^2), and diabetes or hypertension in first degree relatives. Each subject gave written informed consent, and the study was approved by the local ethics committee.

Experiment 1

Each subject underwent a hypoglycemic clamp condition and a euglycemic clamp condition separated by an interval of at least 4 weeks. The order of conditions was balanced across subjects, and experiments were performed in a single-blind fashion. Subjects were randomly assigned to 2 different groups each including 15 subjects. In 1 group, insulin was infused at a rate of 1.5 mU min^{-1}kg^{-1} during both clamps (low insulin), while in the other, the rate of insulin infusion was 15.0 mU min^{-1} kg^{-1} during both clamps (high insulin). Subjects receiving the low insulin rate had a mean age (± SEM) of 26.0 ± 1.0 years (range, 22 to 32 years), those of the high insulin condition had a mean age of 25.4 ± 0.6 years (range, 23 to 29 years).

All subjects were requested to abstain from alcohol, not to perform

From the Departments of Internal Medicine I and Neuroendocrinology, University of Luebeck, Luebeck, Germany.

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Address reprint requests to Bernd Schultes, MD, Medical University Luebeck, Department of Internal Medicine I, Ratzeburger Allee 160, D-23538 Luebeck, Germany.

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any kind of exhausting physical activity, and to go to bed no later than 10 PM on the day preceding the study. On the day of the study, subjects reported to the medical research unit at 8 AM after an overnight fast of at least 10 hours. The experiments took place in a sound-attenuated room with the subject sitting with his trunk in an almost upright position (about 60°) and his legs in a horizontal position on a bed. A cannula was inserted into a vein on the back of the hand, which was placed in a heated box (50°C to 55°C) to obtain arterialized venous blood. A second cannula was inserted into an antecubital vein of the contralateral arm. Both cannulas were connected to long thin tubes, which enabled blood sampling and adjustment of the rate of glucose infusion from an adjacent room without notice of the subject. After a 1-hour baseline period, insulin (H-insulin; Hoechst Marion Roussel, Frankfurt, Germany) was infused at a continuous rates of 1.5 mU $min^{-1}kg^{-1}$ and 15.0 mU $min^{-1}kg^{-1}$, respectively, depending on the group. A 20% glucose solution was simultaneously infused at a variable rate to control plasma glucose levels. Arterialized blood was drawn at 5-minute intervals to measure plasma glucose concentration (Glucose Analyser; Beckman Coulter, Munich, Germany). During the euglycemic clamps, plasma glucose was held stable between 5.0 and 5.5 mmol/L over a period of 360 minutes. During the hypoglycemic clamps, plasma glucose levels were reduced in a stepwise manner to achieve 4 respective plateaus of 4.1, 3.6, 3.1, and 2.6 mmol/L. Each plateau was maintained for a 45-minute period, and the next lower plateau was induced gradually within the next 45 minutes. Blood samples for determination of serum levels of insulin, TSH, fT_3, fT_4, and thyroxine-binding globulin (TBG) were collected every 30 minutes.

Experiment 2

Subjects of experiment 2 had a mean age of 26.7 ± 0.9 years (range, 21 to 33 years). They reported to the medical research unit at 8 AM and then underwent 2 consecutive hypoglycemic clamps, 1 in the morning and another in the afternoon. Subjects were informed not to have breakfast on this day and to abstain from eating until the end of both clamps. The setting of the experiment was identical to that of experiment 1, and the glucose clamp technique was performed in the same way with slight differences in the time course. After a 30-minute baseline period starting at 9 AM, plasma glucose was lowered to a plateau of 2.8 mmol/L by infusion of insulin at a rate of 1.5 mU $min^{-1}kg^{-1}$. Ninety minutes after the start of insulin infusion (11 AM), plasma glucose was further decreased to a plateau of 2.5 mmol/L for another 60 minutes by increasing the rate of insulin infusion to 2.0 mU $min^{-1}kg^{-1}$. At noon, the insulin infusion was stopped, and plasma glucose levels were returned to normal by glucose infusion. At 1 PM, insulin infusion was started again at a rate of 2.0 mU $min^{-1}kg^{-1}$ to induce the second hypoglycemia. During this hypoglycemic clamp, a plasma glucose plateau of 2.5 mmol/L was achieved for 90 minutes (until 3 PM). After the clamp, the subject went home. He was allowed to eat during this time until 10 PM. On the next day (day 2), subjects again reported to the research unit at 8:30 AM for a further examination. Blood was sampled for determination of serum TSH, fT_3, and fT_4 concentrations during the baseline period of day 1 (9 AM), at the end of the first hypoglycemic clamp (noon), and on the morning of day 2 (9 AM). To assess whether potential changes in thyroid hormone levels are associated with changes in REE, indirect calorimetry was performed during the baseline period of day 1 and on day 2 following a standard protocol.[13] Respiratory gas exchanges ($\dot{V}O_2$ and VCO_2) were measured by placing a ventilated hood (MBM-200 Deltatrac II; Datex, Helsinki, Finland) over the subject's head for 10 minutes, while the subject was lying on a bed in a horizontal position.

Analytical Methods

Blood samples were immediately centrifuged and the supernatants stored at -24°C until assay. Serum insulin was measured by radioimmunoassay (Pharmacia Insulin RIA 100; Pharmacia Diagnostics, Uppsala, Sweden) with an intra-assay coefficient of variation (CV) of 5.4% and an interassay CV of 7.5%. Serum concentrations of fT_3 and fT_4, respectively, were measured by enzyme immunoassays (Boehringer Mannheim Immunodiagnostica, Mannheim, Germany) with reference ranges of 4.5 to 9.0 pmol/L for fT_3 (intra-assay CV, 2.3%; interassay CV, 4.7 %) and 10 to 25 pmol/L for fT_4 (intra-assay CV, 1.7%; interassay CV, 3.3 %). Serum TSH concentration was measured by using an immunoluminometric assay (Brahms Diagnostica, Berlin, Germany) with a reference range of 0.2 to 3.5 mU/L (intra-assay CV, 2.2%; inter-assay CV, 2.8%). Serum TBG was measured by using an enzyme immunoassay (Immulite TBG; Diagnostic Products, Los Angeles, CA) with a reference range of 240.5 to 721.5 nmol/L (intra-assay CV, 9.2%; interassay CV, 11.0%). All samples obtained in experiment 1 from each single subject and all samples obtained in experiment 2 from all subjects were run in the same assay.

Statistical Methods

All values are presented as mean ± SEM. Statistical analysis of experiment 1 was based on analysis of variance (ANOVA). ANOVA included the repeated measures factors 'time' (representing the multiple measurements during the clamps), 'hypoglycemia' (for the effects of hypoglycemia v euglycemia), and the between-subjects factor 'insulin' (for the low v high insulin conditions). When main effects for the 'hypoglycemia' factor and the 'hypoglycemia × time' interaction term reached significance ($P < .05$), post hoc analysis was performed separately at each point in time during the clamp to specify the exact time of the effect. In this analysis, baseline values (0 minute) were included as covariate to increase statistical power. Data analysis of experiment 2 was based on Student's paired t test. A P value less than .05 was considered significant.

RESULTS

Experiment 1

In both the high and the low insulin condition, plasma glucose concentration remained stable (5.0 to 5.5 mmol/L) during the euglycemic clamps and decreased stepwise during the hypoglycemic clamps with the pattern of decrease closely comparable between conditions (Fig 1). Serum insulin concentrations reached during the high insulin infusion rate were about 40-fold higher than those during the low rate of insulin infusion (hypoglycemic clamps, 23,624 ± 1,587 v 622 ± 32 pmol/L; euglycemic clamps, 24,029 ± 1,595 v 543 ± 34 pmol/L).

Compared with the euglycemic clamps, serum TSH levels significantly decreased during the hypoglycemic clamps ($P < .001$; Fig 1). The effect did not depend on the rate of insulin infusion ($P = .517$, for the respective interaction term). Separate analysis of the high and low insulin condition showed identical results indicating that serum TSH concentration decreased by 31% ± 3% ($P < .001$) and by 24% ± 5% ($P < .001$) during hypoglycemia, respectively. As shown by comparison at single points in time during the clamps, serum TSH levels started to differ significantly between the euglycemic and hypoglycemic conditions after 240 minutes, ie, when plasma glucose concentration was about 3.2 mmol/L.

Neither hypoglycemia nor the rate of insulin infusion had any effect on serum fT_3 or fT_4 concentration (all $P > .1$, Fig 1). Accordingly, the fT_3/fT_4 ratio was also not influenced by hypoglycemia ($P = .517$) or insulin ($P = .148$). Also, serum TBG levels remained unchanged during all clamps.

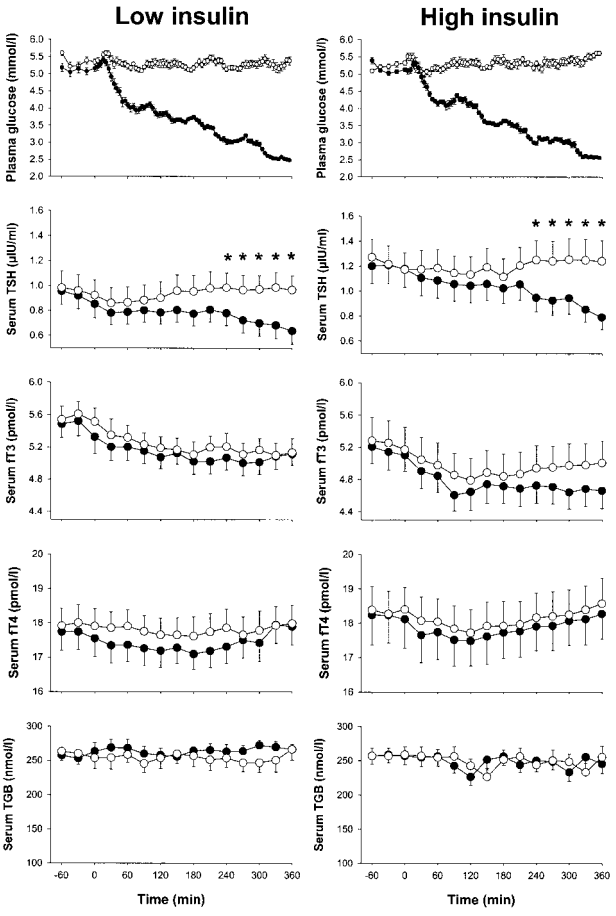

Fig 1. Mean ± SEM plasma glucose concentration and serum concentration of TSH, fT_3, fT_4, and TBG during the euglycemic (○) and stepwise hypoglycemic (●) clamp condition of experiment 1. *$P < .05$ for pairwise comparisons between the effects of conditions.

Experiment 2

The time course of plasma glucose concentration during the clamps performed on day 1 is shown in Fig 2. Plasma glucose dropped below 3.0 mmol/L within 50 minutes after starting the insulin infusion and remained below that level for the next 110 minutes (first hypoglycemia). Thereafter, plasma glucose was increased to euglycemic levels for about 60 minutes and then decreased again to approximately 2.5 mmol/L for another 100 minutes (second hypoglycemia).

Results on hormone measurements are summarized in Fig 3. In response to the first hypoglycemia, serum TSH concentration decreased from 1.28 ± 0.15 to 0.83 ± 0.10 µIU/mL ($P < .001$). In the morning of the next day, serum TSH concentration (0.72 ± 0.10 µIU/mL) was still lower than at the same time on the preceding day ($P < .001$) and also tended to be lower than at the end of the first hypoglycemia of day 1 ($P = .073$). Changes in serum fT_3 concentration during the experiment were quite similar to those observed in TSH levels. Serum fT_3 concentration decreased from 4.78 ± 0.21 to 4.53 ± 0.21 pmol/L during the first hypoglycemia ($P = .004$) and in the morning of the next day was still lower (4.23 ± 0.12 pmol/L) than during the baseline period of the preceding day ($P < .001$). The value of day 2 was also significantly lower than that at the end of first hypoglycemia of day 1 ($P = .024$). In contrast to TSH and fT_3 levels, serum fT_4 concentration slightly increased from 15.2 ± 0.7 to 15.5 ± 0.7 pmol/L during the first hypoglycemia ($P = .064$). However, in the morning of the next day, fT_4 concentration (13.7 ± 0.6 pmol/L) was distinctly lower than during the baseline period of day 1 ($P < .001$) and also lower than at the end of the first hypoglycemia of day 1 ($P < .001$). Corresponding with the changes in fT_3 and fT_4, the fT_3/fT_4 ratio decreased from 0.324 ± 0.015 to 0.294 ± 0.017 during the first hypoglycemia ($P = .006$), but the value in the morning of the next day (0.315 ± 0.015) did not differ from the value measured at the baseline period of day 1 ($P = .172$). There was no difference in $\dot{V}O_2$, $\dot{V}CO_2$, respiratory quotient (RQ), and REE measured respectively in the morning before and after the 2 hypoglycemic episodes (Table 1).

DISCUSSION

To our knowledge, this study for the first time assessed acute effects of hypoglycemia on secretory activity of the pituitary-thyroid axis. Hypoglycemia at a level of 3.2 mmol/L was found to acutely reduce pituitary TSH release without concomitant changes in fT_3 and fT_4 levels. The latter may be explained by the long half-life of these thyroid hormones. However, in experiment 2, more severe hypoglycemia with plasma glucose levels below 3.0 mmol/L for about 110 minutes were found to decrease fT_3 concentration in addition to its lowering effect on TSH concentration. Moreover, 18 hours after 2 episodes of hypoglycemia, serum TSH, fT_3, and fT_4 concentrations were still lower than at the same time of day before the hypoglycemic episodes. Together, the data indicate a prolonged inhibitory influence of hypoglycemic stress on secretory activity of the pituitary-thyroid axis, which outlasts acute hypoglycemia for more than 18 hours.

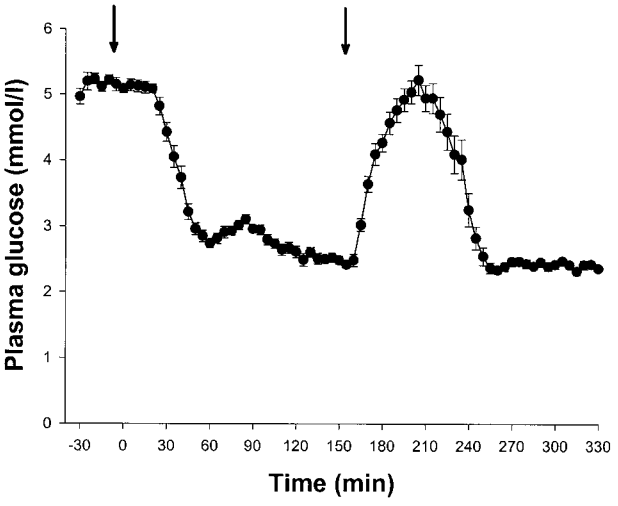

Fig 2. Mean ± SEM plasma glucose concentration during the hypoglycemic clamps performed at day 1 of experiment 2. Arrows indicate the points in time when blood was sampled.

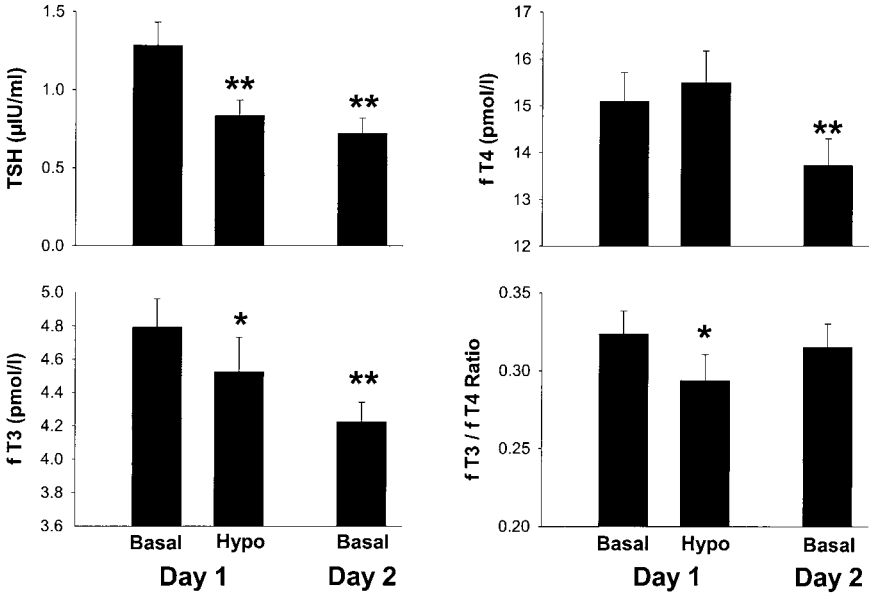

Fig 3. Mean ± SEM serum concentration of TSH, fT_3, and fT_4, as well as the fT_3/fT_4 ratio measured before (basal) and at the end of the first hypoglycemic clamp (hypo) performed at day 1 of experiment 2 and in the morning of the following day (basal, day 2). *$P < .01$, **$P < .001$ for pairwise comparison with the basal values at day 1.

The decrease in serum TSH concentration during hypoglycemia demonstrates that hypoglycemia exerts acutely a suppressing action on pituitary TSH release. From the data of experiment 1, the plasma glucose threshold for this suppressive action can be estimated to be at about 3.2 mmol/L. Lower plasma glucose levels (less than 3.0 mmol/L) maintained for a longer duration as in experiment 2 additionally caused a decrease in serum fT_3 concentration and in the fT_3/fT_4 ratio. This finding suggests that hypoglycemia, in addition to its inhibitory influence on a central, ie, the pituitary and/or hypothalamic, site of the axis, acutely affects peripheral thyroid hormone metabolism by reducing the conversion of T_4 to T_3.

Eighteen hours after the 2 hypoglycemic episodes, serum levels of TSH, fT_3, and fT_4 were still decreased in comparison to concentrations before hypoglycemia, while the fT_3/fT_4 ratio was unchanged. This pattern indicates that the inhibition of pituitary-thyroid secretory activity after antecedent hypoglycemia is prolonged, while the effects on peripheral thyroid hormone metabolism are only of short duration. Taken together, the effects of hypoglycemic stress on the pituitary-thyroid axis obviously differ from the effects of other stressors, such as severe illness or surgery, since the latter in the acute phase are primarily characterized by an alteration in peripheral thyroid hormone metabolism rather than by an inhibition of pituitary TSH release.[4-6] Therefore, one may conclude that hypoglycemia exerts a specific influence on the pituitary-thyroid secretory activity differing from the general type of stress response of the axis. In this context, it should be pointed out that this influence of hypoglycemia on pituitary-thyroid secretory activity could also be mediated by an activation of other neuroendocrine, eg, hypothalamic-pituitary-adrenal or somatotropic, axis and may, therefore, not necessarily represent a direct effect of low plasma glucose concentration.

Table 1. Values of REE, RQ, $\dot{V}o_2$, and $\dot{V}co_2$ Measured by Indirect Calorimetry on 2 Consecutive Days, Before and 18 Hours After Two Hypoglycemic Clamps

	Day 1 Before Hypoglycemia	Day 2 After Hypoglycemia	*P* Value
REE (kcal/d)	1,785 ± 36	1,775 ± 56	.875
RQ	0.81 ± 0.02	0.83 ± 0.02	.389
$\dot{V}o_2$ (mL/min)	261 ± 5	259 ± 8	.819
$\dot{V}co_2$ (mL/min)	211 ± 6	215 ± 7	.709

NOTE. Values are mean ± SEM.

Different rates of insulin infusion were found not to affect serum TSH, fT_3, and fT_4 responses to the euglycemic and hypoglycemic clamps, suggesting that different levels of hyperinsulemia do not influence pituitary-thyroid secretory activity. However, while serum insulin levels in the low insulin condition were within the upper physiologic range, those in the high insulin condition were clearly pharmacologic. Thus, the present result does not exclude the possibility that serum insulin levels within the physiologic range may influence pituitary-thyroid secretory activity.

The clinical relevance of the present findings remains obscure for several reasons. First, prolonged insulin-induced hypoglycemia is not a situation commonly found in clinical practice. Second, the hormonal changes induced by the hypoglycemic clamps were of moderate size and concentrations remained within the physiologic range. Third, the decrease in serum TSH and thyroid hormone concentrations after hypoglycemia were not found to be accompanied by any change in REE. However, the observed phenomenon of acute and prolonged reduction of pituitary-thyroid secretory activity after hypoglycemia may provide new insights into the complex regulation of thyroidal axis function.

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REFERENCES

1. De Groot LJ: Dangerous dogmas in medicine: The nonthyroidal illness syndrome. J Clin Endocrinol Metab 84:151-164, 1999

2. Utiger RD: Altered thyroid function in nonthyroidal illness and surgery. To treat or not to treat? N Engl J Med 333:1562-1563, 1995

3. Kelly GS: Peripheral metabolism of thyroid hormones: A review. Altern Med Rev 5:306-333, 2000

4. Van den Berghe G, de Zegher F, Bouillon R: Clinical review 95: Acute and prolonged critical illness as different neuroendocrine paradigms. J Clin Endocrinol Metab 83:1827-1834, 1998

5. Van den Berghe G: Novel insights into the neuroendocrinology of critical illness. Eur J Endocrinol 143:1-13, 2000

6. Chopra IJ: Clinical review 86: Euthyroid sick syndrome: Is it a misnomer? J Clin Endocrinol Metab 82:329-334, 1997

7. Mitrakou A, Ryan C, Veneman T, et al: Hierarchy of glycemic thresholds for counterregulatory hormone secretion, symptoms, and cerebral dysfunction. Am J Physiol 260:E67-74, 1991

8. Oltmanns KM, Fruehwald-Schultes B, Kern W, et al: Hypoglycemia, but not insulin, acutely decreases lh and t secretion in men. J Clin Endocrinol Metab 86:4913-4919, 2001

9. Tack CJ, Lenders JW, Willemsen JJ, et al: Insulin stimulates epinephrine release under euglycemic conditions in humans. Metabolism 47:243-249, 1998

10. Fruehwald-Schultes B, Kern W, Bong W, et al: Supraphysiological hyperinsulinemia acutely increases hypothalamic-pituitary-adrenal secretory activity in humans. J Clin Endocrinol Metab 84:3041-3046, 1999

11. Davis MR, Mellmann M, Shamoon H: Physiologic hyperinsulinemia enhances counterregulatory hormone responses to hyperglycemia in IDDM. J Clin Endocrinol Metab 76:1383-1385, 1993

12. Davis SN, Shavers C, Collins L, et al: Effects of physiological hyperinsulinemia on counterregulatory response to prolonged hypoglycemia in normal humans. Am J Physiol 267:E402-410, 1994

13. Avignon A, Lapinski H, Rabasa-Lhoret R, et al: Energy metabolism and substrates oxidative patterns in type 2 diabetic patients treated with sulphonylurea alone or in combination with metformin. Diabetes Obes Metab 2:229-235, 2000