Early Changes in Plasma Glucagon and Growth Hormone Response to Oral Glucose in Experimental Hyperthyroidism

Flavia Tosi, Paolo Moghetti, Roberto Castello, Carlo Negri, Enzo Bonora, and Michele Muggeo

The mechanisms underlying deterioration of glucose tolerance associated with hyperthyroidism are not completely understood. Increases in glucagon and growth hormone (GH) secretion have been previously found in hyperthyroid subjects, and could play a crucial role in this phenomenon. However, studies have not yet established the time sequence of changes in plasma glucose on the one hand and glucagon and GH on the other. To assess the early effects of thyroid hormone excess on glucose tolerance and plasma concentrations of the main glucoregulatory hormones, 12 nondiabetic euthyroid subjects underwent an oral glucose tolerance test (OGTT) before and after triiodothyronine ([T_3] 120 μg/d) was administered for 10 days. Plasma levels of glucose, insulin, glucagon, and GH were determined at fasting and after the glucose load. T_3 administration caused a marked increase in serum T_3 (8.8 ± 0.6 *v* 2.0 ± 0.1 nmol/L), with clinical and biochemical signs of thyrotoxicosis. During the treatment, plasma glucose significantly increased both at fasting and after the glucose load (basal, 5.3 ± 0.1 *v* 4.9 ± 0.2 mmol/L, $P < .05$; area under the curve [AUC] for OGTT, 7.7 ± 0.3 *v* 6.7 ± 0.4 mmol/L · min, $P < .01$) without any change in plasma insulin levels. After T_3 administration, plasma glucagon levels were lower than at baseline (basal, 92 ± 7 *v* 148 ± 35 ng/L; AUC, 74 ± 6 *v* 98 ± 16 ng/L · min, $P < .05$), showing an appropriate reduction by the increased glucose levels. Conversely, plasma GH showed impaired suppression by hyperglycemia (AUC, 1.2 ± 0.3 *v* 0.7 ± 0.2 μg/L · min, $P < .05$). In conclusion, thyroid hormone excess rapidly impairs glucose tolerance. Altered secretion of GH is an early event in thyrotoxicosis accompanying the onset of hyperglycemia, whereas plasma glucagon is appropriately suppressed by the increased plasma glucose levels. Thus, GH but not glucagon may contribute to the early hyperglycemic effect of thyrotoxicosis.

ABNORMAL GLUCOSE tolerance is often observed in hyperthyroid patients and usually reverts to normal on attaining euthyroid status.[1-4] To account for these clinical observations, several mechanisms have been proposed, but the pathogenesis of this phenomenon is not completely understood.

Thyroid hormones are capable of directly modulating the activity of several glycometabolic enzymes.[5-9] Nevertheless, derangement of intermediary metabolism in hyperthyroidism is probably mediated, at least in part, by thyroid hormone–induced changes in secretion and/or tissue sensitivity to other glucoregulatory hormones.[10-13]

Conflicting results have been obtained regarding the pattern of insulin secretion and action in hyperthyroid subjects.[2,14-22] The reasons for these discrepancies and the possible role of these alterations remain controversial. Hyperglucagonemia has been consistently reported in hyperthyroid subjects.[10,11,15,23,24] This finding could, in part, account for the increased hepatic glucose production found in thyrotoxicosis.[16,19,25] On this basis, changes in plasma glucagon have been causally related to the impaired glucose tolerance of this condition. Nevertheless, the effective role of glucagon has not been demonstrated.

Thyroid hormones play a major role in the regulation of growth hormone (GH) synthesis and secretion.[26,27] Consistently, enhanced basal GH secretion and impaired GH response to various stimuli have been described in hyperthyroidism.[12,15,28-31] This phenomenon also could be of some relevance in the alteration of glucose tolerance, since GH has a considerable influence on glucose metabolism.[32,33] The role of this factor has not been explored.

This study was undertaken to evaluate the early changes induced by thyroid hormone excess in plasma levels of insulin, glucagon, and GH, with the aim of gaining insight into the pathogenesis of impaired glucose tolerance in hyperthyroidism.

SUBJECTS AND METHODS

Subjects

Twelve healthy subjects, 11 women and one man, were studied after providing informed consent.

Table 1 shows the main characteristics of these subjects. Age ranged from 21 to 52 years, and body mass index from 17.5 to 33.4 kg/m^2. At baseline, all subjects had normal thyroid hormone levels and normal glucose tolerance. None had a history of endocrine or metabolic diseases. No subject was taking any medication during the 3 months before the study.

Procedures

Each subject underwent an oral glucose tolerance test (OGTT) before and after being rendered hyperthyroid by oral administration of triiodothyronine ([T_3] Ti-tre; Glaxo, Greenford, UK) 40 μg three times daily for 10 consecutive days. The test was performed in the morning after an overnight fast, according to National Diabetes Data Group recommendations.[34]

Blood was sampled from a forearm vein via an indwelling butterfly needle before and 30, 60, 90, and 120 minutes after glucose ingestion. Blood samples were assayed for plasma glucose, insulin, glucagon, and GH. Blood for glucagon assay was collected in EDTA tubes containing aprotinin 1,000 KIU/mL. All tubes were placed in ice and promptly centrifuged at 4°C at the end of the test. Samples for hormone measurements were stored at −20°C until the assays were performed.

From the Division of Endocrinology and Metabolic Diseases, University of Verona, Ospedale Maggiore, Verona, Italy.

Submitted November 8, 1995; accepted February 18, 1996.

Supported by grants from the Consiglio Nazionale delle Ricerche (inv 963660) and the Italian Ministry of Higher Education and Scientific Research.

Address reprint requests to Flavia Tosi, MD, Divisone Clinicizzata di Endocrinologia e Malattie del Metabolismo, Ospedale Maggiore, I-37126 Verona, Italy.

0026-0495/96/4508-0020$03.00/0

Table 1. Clinical and Hormonal Characteristics of the Subjects (mean ± SEM)

	Age (yr)	Weight (kg)	Heart Rate (bpm)	T_3 (nmol/L)	T_4 (nmol/L)	FT_4 (pmol/L)	Thyrotropin (mU/L)
Before T_3	39.6 ± 2.4	63.1 ± 3.4	70 ± 4	2.0 ± 0.1	110 ± 5	18.4 ± 0.8	1.1 ± 0.3
After T_3		61.8 ± 3.7*	85 ± 5*	8.8 ± 0.6*	72 ± 5*	13.9 ± 1.3*	<0.1*

*$P < .01$.

Assays

Plasma glucose level was measured by the glucose oxidase method on a Beckman Autoanalyzer (Beckman Instruments, Fullerton, CA). Plasma insulin and glucagon were assayed using double-antibody radioimmunoassay kits (Sorin Biomedica, Saluggia, Italy, and Byk Sangtec, Dietzenbach, Germany, respectively). Cross-reactivity of proinsulin in the insulin kit was 21.4%. Plasma GH level was measured by immunoradiometric assay using kits from Nichols Institute (San Juan Capistrano, CA). Intraassay and interassay coefficients of variation were, respectively, 3.6% and 6.5% for insulin, 3.5% and 4.0% for glucagon, and 2.4% and 3.5% for GH. Sensitivity limits of the assays for glucagon and GH were 35 ng/L and 0.05 μg/L, respectively. All samples from each subject were run in the same assay in duplicate.

Statistics and Calculations

Statistical analyses were performed with a paired Student's *t* test and linear regression. Areas under the plasma concentration curves (AUC) during OGTT were calculated using the trapezoidal method. Values are presented as the mean ± SEM.

RESULTS

T_3 administration caused moderate to severe thyrotoxicosis, as indicated by clinical and biochemical parameters (Table 1). All subjects experienced mild symptoms of nervousness or palpitations. Mean resting heart rate increased by 21%, and mean body weight decreased by 1.0 kg. Plasma T_3 was enhanced fourfold and plasma thyrotropin was decreased to an undetectable level by the short-term thyroid hormone administration.

Glucose Tolerance

In all subjects, glucose tolerance was normal before T_3 administration. Fasting plasma glucose, as well as the plasma glucose response to an oral glucose load, was significantly higher after thyrotoxicosis was induced (basal, 5.3 ± 0.1 *v* 4.9 ± 0.2 mmol/L, $P < .05$; AUC, 7.7 ± 0.3 *v* 6.7 ± 0.4 mmol/L · min, $P < .01$; Fig 1A). One subject showed a diabetic curve after administration of thyroid hormones.

Glucoregulatory Hormones

No difference was noted in fasting or glucose-stimulated plasma insulin levels before and after treatment (Fig 1B).

After T_3, fasting plasma glucagon was decreased, but the difference failed to reach statistical significance. Moreover, glucagon levels after glucose ingestion were significantly lower in the thyrotoxic state than at baseline (Fig 2A).

Fasting plasma GH levels apparently were not affected by thyroid hormone excess. On the other hand, GH suppression following the glucose load was impaired after thyrotoxicosis was induced (Fig 2B).

No relationship was found between serum thyroid hormone levels and basal plasma glucose, insulin, glucagon, and GH levels or their responses to the oral glucose load. Fasting plasma GH after T_3 was positively correlated with the change in plasma glucose AUC induced by thyrotoxicosis ($r = .67, P = .03$).

DISCUSSION

The present study examined the early impact of T_3-induced thyrotoxicosis on glucose tolerance and plasma levels of some major glucoregulatory hormones: insulin, glucagon, and GH. In accordance with previous data,[2,10,20,21]

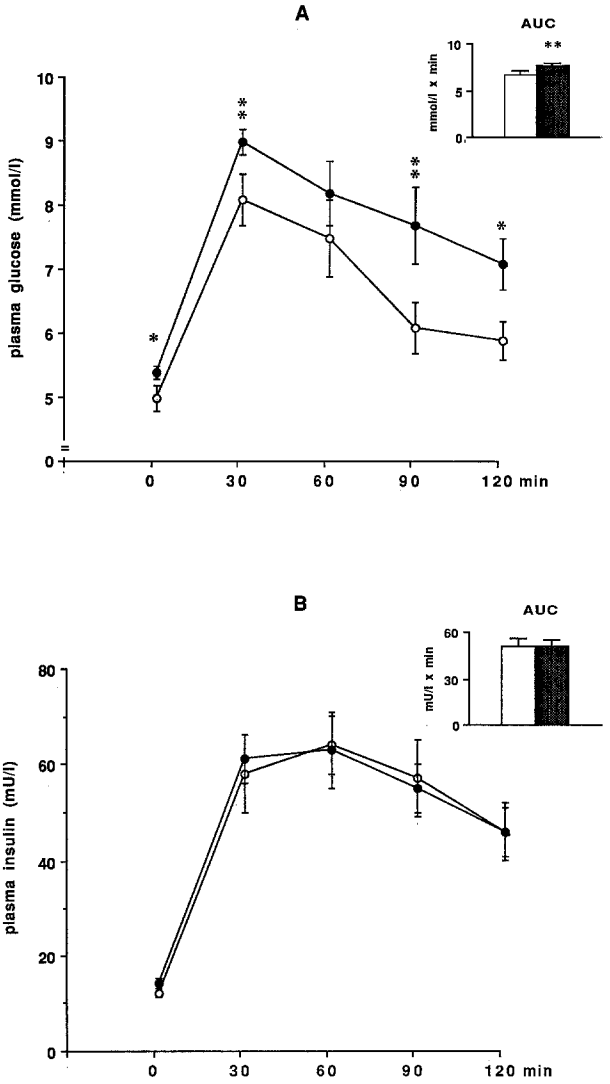

Fig 1. Plasma glucose (A) and insulin (B) levels during OGTT performed before (○) and after (●) T_3 treatment. Inset: AUC before (□) and after (■) treatment. *$P < .05$, **$P < .01$.

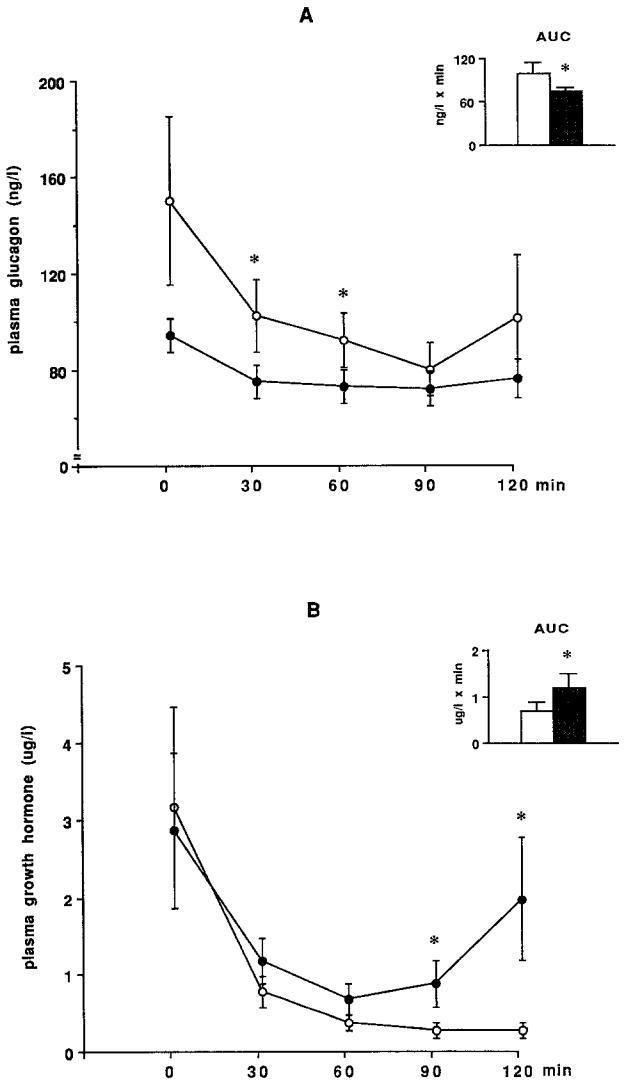

Fig 2. Plasma glucagon (A) and GH (B) levels during OGTT performed before (○) and after (●) T_3 treatment. Inset: AUC before (□) and after (■) treatment. *$P < .05$.

our results showed that even a short-term thyroid hormone excess is capable of impairing glucose tolerance: T_3 administration increased mean plasma glucose by approximately 1 mmol/L over baseline values throughout the OGTT; remarkably, one subject became diabetic after T_3 treatment.

Despite higher plasma glucose, no change in basal and stimulated plasma insulin concentrations was observed after T_3. This finding suggests a relative impairment in glucose-induced insulin secretion and/or an enhancement in insulin clearance. With respect to these hypotheses, data from previous studies yielded conflicting results. Fasting plasma insulin and its response to oral glucose have been reported to be increased, normal, or decreased in hyperthyroidism.[2,18-21] It is noteworthy that hyperinsulinemia, often found in hyperthyroid patients, may be attributable, at least in part, to increased plasma proinsulin,[13,35,36] which cross-reacts with anti-insulin antibodies in commonly used kits for insulin assay. In assessing insulin secretion by C-peptide levels, some studies reported impaired insulin secretion in hyperthyroid patients.[18,20,36] This hypothesis has also been supported by the finding of low insulin content in pancreatic islets of hyperthyroid rats.[37] The role of possible β-cell dysfunction is unclear. Evaluation of insulin clearance also gave controversial results. However, most of the studies examining this parameter found no alteration in hyperthyroidism.[13,16,19,22] It should be noted that in absolute terms plasma insulin was unchanged after T_3 in our subjects. This makes it unlikely that any impairment of insulin secretion may be the sole cause of hyperglycemia, although it may be a contributory factor.

In contrast to findings in spontaneous chronic hyperthyroidism, our data in the early thyrotoxic condition failed to demonstrate increased glucagon levels. On the contrary, plasma concentrations of this hormone were appropriately decreased by the higher plasma glucose levels. Since hyperglycemia precedes the increase in glucagon levels, it could be argued that glucagon is not the factor determining the impairment in glucose tolerance associated with thyroid hormone excess. Nevertheless, when plasma glucagon has increased later in the natural history of spontaneous hyperthyroidism,[10,11,15,23,24] effects of hyperglucagonemia might also contribute to alterations of glucose metabolism.

From this study, it can also be concluded that hyperglucagonemia does not seem to be a direct effect of thyroid hormone excess. A possible explanation for this phenomenon in chronic hyperthyroidism is hepatic glycogen depletion,[3,38,39] which represents the main stimulus for glucagon secretion.[40,41] Glucagon has been demonstrated to promote thyroxine (T_4) conversion to rT3[42] and to reduce the number of thyroid hormone receptors in animal models.[43] We can thus speculate that hyperglucagonemia from prolonged hyperthyroidism might also be an adaptation to reduce the impact of thyroid hormone hypersecretion.

This study did not show changes in fasting plasma concentrations of GH in the thyrotoxic condition. However, a single determination of GH levels is inadequate to assess basal secretion of this hormone, which requires frequent blood sampling throughout the entire day to document secretory bursts. This was not possible in the present study. Indeed, several studies reported no significant difference in basal GH levels between hyperthyroid patients and controls,[15,23,28,30,31] although increased spontaneous hormone secretion has been clearly demonstrated in hyperthyroidism.[12] According to previous reports in spontaneous hyperthyroidism,[28,44,45] after short-term administration of T_3 there was a blunted inhibitory effect of hyperglycemia on GH levels. Altered secretion of GH thus seems to be an early event in thyrotoxicosis, accompanying the onset of hyperglycemia. This hormone undoubtedly exerts insulin-antagonistic activity on glucose metabolism. GH hyperglycemic effects of clinical significance have been demonstrated in acromegaly[46] and diabetes,[47] as well as in GH-deficient[48] or normal[32] subjects given exogenous GH. Furthermore, GH infusion acutely induces pronounced and very early decreases in peripheral glucose uptake.[33] In the present study, a relationship was found between fasting GH levels after T_3 administration and the increase in plasma glucose levels

induced by thyroid hormone excess. As a whole, these observations suggest that GH may also play a pathogenetic role in impaired glucose tolerance from hyperthyroidism. However, in this case, increased insulin levels would be expected. A plausible explanation for the absence of hyperinsulinemia would be that insulin secretion is also impaired. Further studies are required to confirm this hypothesis.

In conclusion, thyrotoxicosis rapidly impairs glucose tolerance. GH but not glucagon may contribute to the early hyperglycemic effect of thyroid hormone excess.

ACKNOWLEDGMENT

We thank Alessandra Rossi and M. Grazia Zanotti for excellent technical assistance.

REFERENCES

1. Kreines K, Jett M, Knowles HC: Observations in hyperthyroidism of abnormal glucose tolerance and other traits related to diabetes mellitus. Diabetes 14:740-744, 1965

2. Doar JWH, Stamp TCB, Wynn V, et al: Effects of oral and intravenous glucose loading in thyrotoxicosis. Diabetes 18:633-639, 1969

3. Andreani D, Menzinger G, Fallucca F, et al: Insulin levels in thyrotoxicosis and primary myxoedema: Response to intravenous glucose and glucagon. Diabetologia 6:1-7, 1970

4. Ahren B: Hyperthyroidism and glucose intolerance. Acta Med Scand 220:5-14, 1986

5. Malbon CC, Campbell R: Thyroid hormones regulate hepatic glycogen synthase. Endocrinology 115:681-686, 1984

6. Dimitriadis GD, Leighton B, Vlachonikolis IG, et al: Effects of hyperthyroidism on the sensitivity of glycolysis and glycogen synthesis to insulin in the soleus muscle of the rat. Biochem J 253:87-92, 1988

7. Celsing F, Blomstrand E, Melichna J, et al: Effect of hyperthyroidism on fibre-type composition, fibre area, glycogen content and enzyme activity in human skeletal muscle. Clin Physiol 6:171-181, 1986

8. Bollen M, Stalmans W: The effect of thyroid status on the activation of glycogen synthase in liver cells. Endocrinology 122: 2915-2919, 1988

9. Lomax RB, Robertson WR: The effects of hypo- and hyperthyroidsm on fibre composition and mitochondrial enzyme activities in rat skeletal muscle. J Endocrinol 133:375-380, 1992

10. Kabadi UM, Eisenstein AB: Glucose intolerance in hyperthyroidism: Role of glucagon. J Clin Endocrinol Metab 50:392-396, 1980

11. Wolf E, Eisenstein AB: Portal vein blood insulin and glucagon are increased in experimental hyperthyroidism. Endocrinology 108:2109-2113, 1981

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

13. O'Meara NM, Blackman JD, Sturis J, et al: Alterations in the kinetics of C-peptide and insulin secretion in hyperthyroidism. J Clin Endocrinol Metab 76:79-84, 1993

14. Muller MJ, von Schutz B, Huhnt HJ, et al: Glucoregulatory function of thyroid hormones: Interaction with insulin depends on the prevailing glucose concentration. J Clin Endocrinol Metab 63:62-71, 1986

15. Moghetti P, Castello R, Tosi F, et al: Glucose counterregulatory response to acute hypoglycemia in hyperthyroid human subjects. J Clin Endocrinol Metab 78:169-173, 1994

16. McCulloch AJ, Home PD, Heine R, et al: Insulin sensitivity in hyperthyroidism: Measurement by the glucose clamp technique. Clin Endocrinol (Oxf) 18:327-333, 1983

17. Randin JP, Tappy L, Scazziga B, et al: Insulin sensitivity and exogenous insulin clearance in Graves' disease. Measurement by the glucose clamp technique and continuous indirect calorimetry. Diabetes 35:178-181, 1986

18. Roti E, Braverman LE, Robuschi G, et al: Basal and glucose- and arginine-stimulated serum concentrations of insulin, C-peptide, and glucagon in hyperthyroid patients. Metabolism 35:337-342, 1986

19. Shen D, Davidson MB, Kuo S, et al: Peripheral and hepatic insulin antagonism in hyperthyroidism. J Clin Endocrinol Metab 66:565-569, 1988

20. Bonora E, Manicardi V, Zenere M, et al: Plasma C-peptide response to oral glucose load in hyperthyroidism. J Endocrinol Invest 13:555-558, 1990

21. Ikeda T, Fujiyama K, Hoshino T, et al: Oral and intravenous glucose-induced insulin secretion in hyperthyroid patients. Metabolism 39:633-637, 1990

22. Pestell R, Alford F, Ramos R, et al: Insulin secretion, insulin sensitivity and glucose-mediated glucose disposal in thyrotoxicosis: A minimal model analysis. Clin Endocrinol (Oxf) 33:481-493, 1990

23. Perez G, Ungaro B, Covelli A, et al: Altered glucoregulatory response to physiological infusions of epinephrine and glucagon in hyperthyroidism. J Clin Endocrinol Metab 51:972-977, 1980

24. Lam KSL, Yeung RTT, Ho PWM, et al: Glucose intolerance in thyrotoxicosis: Roles of insulin, glucagon and somatostatin. Acta Endocrinol (Copenh) 114:228-234, 1987

25. Dimitriadis G, Baker B, Marsh H, et al: Effect of thyroid hormone excess on action, secretion, and metabolism of insulin in humans. Am J Physiol 248:E593-E601, 1985

26. Samuels HH, Forman BM, Horowitz ZD, et al: Regulation of gene expression by thyroid hormones. Annu Rev Physiol 51:623-639, 1989

27. Miki N, Ono M, Hizuka N, et al: Thyroid hormone modulation of hypothalamic growth hormone (GH)-releasing factor–pituitary GH axis in the rat. J Clin Invest 90:113-120, 1992

28. Valcavi R, Dieguez C, Zini M, et al: Influence of hyperthyroidism on growth hormone secretion. Clin Endocrinol (Oxf) 38:515-522, 1993

29. Davies RR, Jack D, Turner SJ, et al: The effect of thyroid status on the growth hormone response to growth hormone releasing hormone 1-44. J Endocrinol Invest 12:517-521, 1989

30. Giustina A, Ferrari C, Bodini C, et al: Effects of methimazole treatment on growth hormone (GH) response to GH-releasing hormone in patients with hyperthyroidism. Acta Endocrinol (Copenh) 123:613-618, 1990

31. Giustina A, Schettino M, Bussi AR, et al: Effect of arginine on the GHRH-stimulated GH secretion in patients with hyperthyroidism. Horm Res 38:256-259, 1992

32. Bratusch-Marrain PR, Smith D, DeFronzo RA: The effect of growth hormone on glucose metabolism and insulin secretion in man. J Clin Endocrinol Metab 55:973-982, 1982

33. Moller N, Jorgensen JOL, Alberti KGMM, et al: Short-term effects of growth hormone on fuel oxidation and regional substrate metabolism in normal man. J Clin Endocrinol Metab 70:1179-1186, 1990

34. National Diabetes Data Group: Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes 28:1039-1057, 1979

35. Sestoft L, Heding LG: Hypersecretion of proinsulin in thyrotoxicosis. Diabetologia 21:103-107, 1981

36. Beer SF, Parr JH, Temple RC, et al: The effect of thyroid disease on proinsulin and C-peptide levels. Clin Endocrinol (Oxf) 30:379-383, 1989

37. Lenzen S, Joost HG, Hasselblatt A: Thyroid function and insulin secretion from the perfused pancreas in the rat. Endocrinology 99:125-129, 1976

38. Pipher J, Poulsen E: Liver biopsy in thyrotoxicosis. Acta Med Scand 127:439-447, 1947

39. Aranda A, Montoya E, Errera E: Effects of hypo and hyperthyroidism on liver composition, blood glucose, ketone bodies and insulin in the male rat. Biochem J 128:597-604, 1972

40. Kabadi UM: Is hepatic glycogen content a regulator of glucagon secretion? Metabolism 41:113-115, 1992

41. Kabadi UM: Hepatic regulation of pancreatic α-cell function. Metabolism 42:535-543, 1993

42. Kabadi UM, Premachandra BN: Decline of T_3 and elevation in reverse T_3 induced by hyperglucagonemia: Changes in thyroid hormone metabolism, not altered release of thyroid hormones. Horm Metab Res 20:513-516, 1988

43. Dillmann WH, Bonner RA, Oppenheimer JH: Glucagon administration decreases hepatic nuclear triiodothyronine binding capacity. Endocrinology 102:1633-1636, 1978

44. Vinik A, Pimstone B, Buchanan-Lee B: Impairment of hyperglycemic induced growth hormone suppression in hyperthyroidism. J Clin Endocrinol 28:1534-1538, 1968

45. Finkelstein JW, Boyar RM, Hellman L: Growth hormone secretion in hyperthyroidism. J Clin Endocrinol Metab 38:634-637, 1974

46. Hansen I, Tsalikian E, Beaufrere B, et al: Insulin resistance in acromegaly: Defects in both hepatic and extrahepatic insulin action. Am J Physiol 250:E269-E273, 1986

47. Press M, Tamborlane MW, Sherwin RS: Importance of raised growth hormone levels in mediating the metabolic derangements of diabetes. N Engl J Med 310:810-815, 1984

48. Salomon F, Cuneo RC, Hesp R, et al: The effects of treatment with recombinant human growth hormone on body composition and metabolism in adults with growth hormone deficiency. N Engl J Med 321:1797-1803, 1989