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# The Effects of Thyroid Hormones on Glucokinase Enzyme Activity in Diabetic Rat Liver Tissues

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## Abstract

**Objective:** We aimed to investigate the effects of insulin and various doses of thyroid hormones with combine treatment on glucokinase (GK) enzyme activity, which is the one of the key enzyme in carbohydrate metabolism, diabetes and hypothyroidism.

**Material and methods:** Rats were assigned to eight groups: Group 1: control, Group 2: diabetes (DM), Group 3: DM + insulin, Group 4: thyroidectomized control, Group 5: thyroidectomized + DM, Group 6: thyroidectomized + DM + insulin, Group 7: thyroidectomized + DM + insulin + thyroid hormone (TH) (2.5µg/kg), Group 8: thyroidectomized + DM + insulin + TH (5 µg/kg). Glucose concentration, HbA1c and thyroid hormone levels were measured in blood samples and GK enzyme activities were determined in liver tissue samples.

**Results:** Our results showed that the glucokinase enzyme activities were significantly decreased in diabetic rat liver tissues compared to control group. Moreover it was observed that the enzyme activities were slightly regulated by insulin. It is demonstrated that decreased GK enzyme activity in thyroidectomized diabetic rat liver was regulated by insulin and various doses of thyroid hormones.

**Conclusion:** As a result the possible contribution of thyroid hormones to insulin effect to normalize diabetic induced changes in liver tissue has been shown.

**Keywords:** Glucokinase, Thyroid hormone, Diabetes Mellitus, Rat liver

## Tiroid Hormonlarının Diyabetik Rat Karaciğer Glikokinaz Enzimi Üzerine Olası Etkileri

## Öz

**Amaç:** Karbonhidrat metabolizması, diyabet ve hipotiroidizmde anahtar enzimlerden biri olan glikokinaz (GK) enzim aktivitesi üzerine insülin ve çeşitli tiroid hormon dozlarının birlikte tedavi ile etkilerini araştırmayı amaçladık.

**Gereç ve yöntem:** Ratlar 8 gruba ayrıldı: Grup 1: Kontrol, Grup 2: Diyabet (DM), Grup 3: DM + insülin, Grup 4: Tiroidektomi, Grup 5: Tiroidektomi + DM, Grup 6: Tiroidektomi + DM + insülin, Grup 7: Tiroidektomi + DM + insulin + tiroid hormonu (TH) (2.5µg/kg), Grup 8: Tiroidektomi + DM + insulin + TH (5µg/kg). Serum örneklerinde glikoz konsantrasyonu, HbA1c ve tiroid hormon düzeyleri ölçüldü, karaciğer doku örneklerinde GK enzim aktivitesi tayin edildi.

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**Bulgular:** Diyabetik ratların karaciğer dokusunda, glikokinaz enzim aktivitesinde kontrol grubuna göre anlamlı bir azalma gözlendi ($p<0,05$). İnsülin tedavisinden sonra bu değişikliklerin bir miktar düzeldiği izlendi. Ayrıca, tiroidektomize diyabetik rat karaciğerinde azalmış GK enzim aktivitesinin insülin ve çeşitli dozlarda tiroid hormonları tarafından düzenlendiği gösterildi.
**Sonuç:** Çalışmamız sonuçlarına dayanarak, diyabetik rat karaciğer dokusunda, glikokinaz enzim aktivitesinde meydana gelen değişikliklerin insülin tedavisi ile normale dönüşünde tiroid hormonlarının olası etkilerinin olabileceği düşünüldü.

**Anahtar Kelimeler:** Glikokinaz, Tiroid hormonu, Diyabet, Rat karaciğeri

## Introduction

Diabetes Mellitus (DM) is a group of metabolic diseases characterized by chronic hyperglycemia resulting from defects in insulin metabolism and impaired function in carbohydrate, lipid and protein metabolism that leads to long-term complications (1). The enzyme glucokinase (GK) (E.C. 2.7.1.2) has a low affinity for glucose and largely expressed in liver and pancreatic beta-cells, playing a key ‘glucose sensing’ role to regulate hepatic glucose balance and insulin secretion, it is also the principle glucose-phosphorylating enzyme in the parenchymal liver cells of mammals. The expression of glucokinase in the rat liver is under multihormonal control. It is induced by insulin at the transcriptional level (2,3) and supressed by glucagon through its second messenger cAMP at a pre-translational level (3-6). After food withdrawal and subsequent refeeding a maximal response in glucokinase activity is only achived in the presence of glucocorticoids and thyroid hormones (7-9).

Induction of diabetes by streptozotocin (stz) has been shown to produce a hypothyroid state in experimental animals (10,11). In different studies it was mentioned that thyroid hormones contribute to the regulation of blood sugar by accelerating the turnover of glucose (12). The mechanism by which thyroid hormones stimulate the rate of glucose utilization in the liver was determined by investigating the effect of different thyroid states on the expression of the glucokinase gene, a key enzyme of glycolysis (13-15). Circulating levels of triiodothyronine are observed with increased carbohydrate consumption, which stimulates the induction of glucokinase activity (9). Moreover, phosphorylation of glucose is the first step in glycolysis and a prerequisite for the conversion of glucose into fatty acids, GK may also be viewed as a lipogenic enzyme. It is well known that, one of the functions of thyroid hormones in the regulation of intermediary metabolism in liver is the induction of a set of enzymes involved in lipogenesis (16-19). Therefore, an effect of thyroid hormones on the induction of GK could be expected. Indeed, the regulation of the expression of this gene by insulin is modulated by thyroid hormones. To sum up, in our study, we aimed to investigate that combine effects of thyroid hormones and insulin in the regulation of impaired glucokinase enzyme activity in liver tissues of streptozotocin diabetic, thyroidectomized and insulin and thyroid hormone treated rats.

## Material and Methods

The study protocol was reviewed and approved by the Animal Care Committee and Surgical Research Center of Gazi University Faculty of Medicine (GUDAM). Guiding principles for experimental procedures found in the Declaration of Helsinki of the World Medical Association regarding animal experimentation were followed in the study. In this study male Sprague-Dawley rats, weighting 200-250 g were maintained with 12 hours of light and dark cycle, controlled humidity, temperature and free access to standard diet and tap water for 7 days prior to experiment. Rats were assigned to eight groups: Group 1: control, Group 2: diabetes (with stz 55mg/kg, intraperitoneally), Group 3: diabetes + insulin (after diabetes induction, rats were treated with insulin for five weeks (7-10 U/kg/day, Insulatard-HM® Penfill®, Novo Nordisk, 100 IU/ml NPH, subcutaneously), Group 4: surgically thyroidectomized control, Group 5: thyroidectomized + diabetes (diabetes was induced 3 weeks after thyroidectomy), Group 6: thyroidectomized + diabetes + insulin (after diabetes inductions, rats were treated with insulin for five weeks (7-10 U/kg/day, subcutaneously), Group 7: thyroidectomized + diabetes + insulin + thyroid hormone (after diabetes induction, rats were treated both with insulin (7-10 U/kg/day, subcutaneously) and thyroid hormone levothyroxin sodium, 2.5 µg/kg, Tefor® (Organon) for five weeks, Group 8; thyroidectomized + diabetes + insulin + thyroid hormone (after diabetes induction, rats were treated both with insulin (7-10 U/kg/day, subcutaneously) and thyroid hormone levothyroxin sodium, 5 µg/kg for five weeks.

Blood glucose concentrations were immediately determined by glucose oxidase enzymatic assay with an Ames Glucometer (Miles Laboratories Inc., Elkhart, IN, USA). The free $T_3$ ($FT_3$), free $T_4$ ($FT_4$), total $T_3$ ($T_3$), and $T_4$ ($T_4$) concentrations were measured in serum samples by otoanalyzer which were a competitive enzyme immunoassay (TOSOH AIA-21, TOSOH Bioscience, N.V., Tessenderlo, Belgium).

Each liver tissue was washed with ice-cold physiological saline and weighed. In order to ensure standard processing, the left lobe was marked and covered with aluminium foil, frozen under liquid nitrogen and kept at -80 °C until processing. GK enzyme activitiy was determined in liver tissue samples according to the spectrophotometric method of Walker and Parry (20) and modifications by Di Pietro and Wienhouse (21). Due to this method, glucose which is phosphorylated by glucokinase, is converted to glucose-6-phosphate. After this reaction glucose-6-phosphate is reduced by glucose-6-phosphate dehydrogenase to use NADP. Reduced $NADP^+$ is measured at 340 nm and the increase in enzyme activity corresponds to glucokinase activity of 1 µmol glucose phosphorylated per minute when only 1 mole of $NADP^+$ is reduced per mole of glucose phosphorylated. One unit of activity is defined as that amount of enzyme catalyzing the phosphorylation of 1 µmol of glucose per minute at 28 °C. Specific activity is expressed as units per milligram of protein. The protein levels were measured by a method of Lowry et al. (22). Tissue GK activity was stated as mU/mg protein.

### Statistical analysis

Statistical analysis was performed using statistical software SPSS for Windows, version 17.0, (SPSS Inc., USA). The p values <0.05 were considered statistically significant for all analyses. All values presented in tables were expressed as mean ± SD. The Kruskal–Wallis (non-parametric) test was applied to evaluate differences among all the groups, while differences between pairs of groups were evaluated by means of the Tukey's post-hoc test.

### Results

After two or three days of stz administration, rats demonstrated polyphagia, polydipsia, polyuria and stable hyperglycemia for 5 weeks which determined by measuring blood glucose levels for every 3 days (> 400 mg/dL) and these symptoms of Diabetes Mellitus were supported by increased levels of HbA1c. Blood glucose concentrations, HbA1c levels and thyroid hormones concentrations [Free $T_3$ ($FT_3$), free $T_4$ ($FT_4$) total $T_3$ ($T_3$) and total $T_4$ ($T_4$)] were given in the previous manuscript (23) and also were shown in Table 1, 2.

**Table 1.** Changes in the body weight, blood glucose concentration and HbA1c of all groups (values represent mean ± SD) (23)

| Groups | | Body Weight (g) | Blood Glucose Concentration (mg/dL) | HbA1c (%) |
|---|---|---|---|---|
| Group 1 (n=10) | Control | 235.0 ± 35.3 | 109.5 ± 4.6 | 4.82 ± 0.30 |
| Group 2 (n=10) | DM | 159.0 ± 32.2[ac] | 424.0 ± 22.8[a] | 12.11 ± 0.60[ac] |
| Group 3 (n=7) | DM+Insulin (I) | 232.1 ± 48.5[bc] | 108.0 ± 18.2[bc] | 4.24 ± 0.07[abc] |
| Group 4 (n=10) | Thyroidectomized (Thy) | 285.0 ± 34.7[abc] | 115.8 ± 20.1[bc] | 4.64 ± 0.20[bc] |
| Group 5 (n=8) | Thy+DM | 235.0 ± 22.7[b] | 487.4 ± 35.7[a] | 6.01 ± 0.80[ab] |
| Group 6 (n=9) | Thy+DM+I | 239.5 ± 32.9[b] | 109.2 ± 9.6[bc] | 5.38 ± 0.80[ab] |
| Group 7 (n=4) | Thy+DM+I+TH (T$_4$, 2.5µg/kg) | 225.0 ± 5.7[b] | 92.7 ± 4.0[bc] | 4.76 ± 0.15[bc] |
| Group 8 (n=6) | Thy+DM+I+TH (T$_4$, 5µg/kg) | 232.3 ± 2.6[b] | 92.7 ± 3.1[bc] | 4.92 ± 0.13[bc] |

n=number of rats

[a]: significant compared to Group 1, control (p<0.05)

[b]: significant compared to Group 2, DM (p<0.05)

[c]: significant compared to Group 5, Thy+DM (p<0.05)

**Table 2.** Serum thyroid hormone levels of all groups (values represent mean ± SD) (23)

| Groups | | FT$_3$ (pg/mL) | FT$_4$ (pmol/L) | T$_3$ (ng/mL) | T$_4$ (nmol/L) |
|---|---|---|---|---|---|
| | Expected Reference Interval for kit | 2.1-3.8 | 9.67-19.86 | 0.78-1.59 | 51.6-141.9 |
| Group 1 (n=10) | Control | 3.87 ± 0.63 [bc] | 19.84 ± 2.15 [bc] | 0.64 ± 0.02 [bc] | 40.11 ± 3.30 [bc] |
| Group 2 (n=10) | DM | 1.39 ± 0.35 [a] | 7.54 ± 3.17 [a] | 0.28 ± 0.07 [a] | 19.47 ± 7.23 [ac] |
| Group 3 (n=7) | DM+I | 4.33 ± 0.77 [bc] | 28.73 ± 4.15 [abc] | 0.41 ± 0.11 [abc] | 35.38 ± 4.06 [abc] |
| Group 4 (n=10) | Thy | 1.46 ± 0.08 [a] | 9.14 ± 2.06 [ac] | 0.29 ± 0.09 [a] | 15.54 ± 4.15 [ac] |
| Group 5 (n=8) | Thy+DM | 1.39 ± 0.26 [a] | 4.20 ± 2.31 [ab] | 0.28 ± 0.05 [a] | 6.54 ± 0.01 [ab] |
| Group 6 (n=9) | Thy+DM+I | 2.63 ± 0.98 [abc] | 7.56 ± 2.12 [a] | 0.26 ± 0.05 [a] | 20.06 ± 5.24 [ac] |
| Group 7 (n=4) | Thy+DM+I+TH (T$_4$, 2.5µg/kg) | 5.10 ± 0.83 [abc] | 26.52 ± 3.00 [abc] | 0.46 ± 0.02 [abc] | 46.27 ± 6.81 [bc] |
| Group 8 (n=6) | Thy+DM+I+TH (T$_4$, 5µg/kg) | 7.05 ± 1.95 [abc] | 21.07 ± 9.48 [bc] | 0.64 ± 0.12 [bc] | 41.78 ± 15.80 [bc] |

n=number of rats

[a]: significant compared to Group 1, control (p<0.05)

[b]: significant compared to Group 2, DM (p<0.05)

[c]: significant compared to Group 5, Thy + DM (p<0.05)

GK enzyme activity significantly decreased in groups 2 compared to group 1 ($p<0.05$), but increased in groups 7, 8 compared to group 1. Enzyme activities were increased ($p<0.05$) in group 6, 7 and 8 compared to group 2 and 5 (Table 3). Enzyme activity of GK was decreased significantly, however this activity was normalized slightly by the treatment of insulin. While insulin treatment surged considerably enzyme activity of GK in trioidectomized diabetic rats, the administiration of thyroid hormones at different doses seemed to substantially rise this effect, especially in high dose group. Because of the GK activity reduced significantly in the absence of insulin, however in the absence of thyroid hormone declined activity of GK was not significant. This situation suggests that the primary control of GK activity is achieved by insulin, but thyroid hormone presence may mediates insulin effect.

## Discussion

We designed our study to observe the possible effects of thyroid hormones on GK enzyme activity of thyroidectomized and stz-induced diabetic rat liver tissues. Between the eight groups of our study, we observed many alterations in GK enzyme activities that we concluded our study suggested the close interactions between thyroid hormones and insulin and glucokinase enzyme activities.

In different studies in humans, animal models, and isolated hepatocytes have established that hepatic GK exerts a very strong influence on glucose utilization and glycogen synthesis (12,14,24). Moreover, complementary studies in primary hepatocytes have shown that GK overexpression elevates intracellular G6P which triggers an increase in both glycolysis and glycogen synthesis (12,24).

**Table 3.** Changes in glucokinase enzyme activities of all groups (values represent mean ± SD)

| Groups | | **GK enzyme activities (mU/mg protein)** |
|---|---|---|
| Group 1 (n=10) | Control | 20.91 ± 4.46 $^{b}$ |
| Group 2 (n=10) | DM | 10.89 ± 2.89 $^{a}$ |
| Group 3 (n=7) | DM+I | 14.69 ± 3.21 |
| Group 4 (n=10) | Thy | 16.59 ± 4.00 |
| Group 5 (n=8) | Thy+DM | 17.74 ± 5.33 |
| Group 6 (n=9) | Thy+DM+I | 30.58 ± 4.54 $^{b,c}$ |
| Group 7 (n=4) | Thy+DM+I+TH ($T_4$, 2.5µg/kg) | 33.86 ± 9.01 $^{a,b,c}$ |
| Group 8 (n=6) | Thy+DM+I+TH ($T_4$, 5µg/kg) | 62.29 ± 15.87 $^{a,b,c}$ |

n=number of rats

$^{a}$: significant compared to Group 1, control ($p<0.05$)

$^{b}$: significant compared to Group 2, DM ($p<0.05$)

$^{c}$: significant compared to Group 5, Thy+DM ($p<0.05$)

The results of different studies mentioned GK in pancreatic islet tissue and proposed that GK functions as the molecular glucose sensor element in the insulin-producing pancreatic-cells in addition to its established function as the high-capacity enzymatic step initiating the storage of glucose in the form of glycogen in the liver (2,25-27).

The expression of GK in the rat liver is under multihormonal control (13,28). It can be induced by insulin at the transcriptional level (2,3) and suppressed by glucagon through its second messenger cAMP at a pretranslational level (3-6,13). The limitation of our study was not to work on mRNA expression of glucokinase however we had recorded increases in GK enzyme activity in the liver tissue after the insulin treatment in group 3 and 6 compared to untreated diabetic groups.

Furthermore,in different studies after food withdrawal and subsequent refeeding a maximal response in GK activity was only achieved in the presence of glucocorticoids and thyroid hormones (3-6,9,28,29) in which glucocorticoids and thyroid hormones were found to be essential factors for the rapid insulin-mediated induction of GK synthesis in vivo. The multiple hormonal regulations can be suggest in GK enzyme activity. However in our study, without thyroid hormone and with half dose of thyroid hormone treatment together with insulin treatment were able to increase the GK enzyme activity but doubled the increasing activity of GK in group 8 after the insulin treatment with full dose treatment of thyroid hormone.

Subphysiological concentrations of $T_3$ also led to considerable increases in GK gene expression, suggesting an important role of thyroid hormones at their physiological circulating concentrations in the modulation of this gene and thereby in the regulation of glucose utilization. $T_3$ exerts its action on glucokinase gene expression exclusively at the level of transcription (13,29). Only a few studies have investigated the effect of thyroid dysfunction and its recovery by thyroid hormone treatment on glucose metabolism, and the results have been controversial (30-33). It is not clear whether thyroid hormone replacement is effective for restoring the insulin secretion. In our study GK enzyme activity decreased in thyroidectomized group. Additionally we think that diabetes-induced hypothyroidism may contribute to decreased GK activity.

In our study, we tried to observe the complete outcomes of diabetes, hypotyroidism and treatments with insulin or two different doses of thyroid hormones or both. Insulin by itself not sufficient to alter the GK enyzme activities in thyroidectomized groups, but depending on the combined treatments with different doses of thyroid hormones, we observed appropriate GK enyzme activities. This suggests that the prolonged absence of one hormone can modulate the cellular responses to other hormones. As a result we can conclude that the normalised diabetic induce changes in liver tissues of glucokinase enzyme activities after the insulin treatment may the depend on the possible contributions of thyroid hormones.

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