Correlation of Insulin Resistance with Thyroid Profile in Streptozotocin Induced Type 2 Diabetic Rats
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Abstract

Background: To assess the association between insulin resistance, thyroid profile and glucose levels in type 2 diabetes mellitus.

Methods: In this randomized control trial, 60 Sprague Dawley rats were divided into control and diabetic groups. All the rats were checked for euglycemia and euthyroidism at the beginning of the study by analyzing plasma glucose and serum thyroid stimulating hormone (TSH) levels. The diabetic group was fed high fat diet while control group was given normal pellet diet. After 2 weeks diabetic rats were intraperitoneally administered low dose of streptozotocin and saline to the control group. Development of type 2 diabetes was confirmed by checking homeostatic model for insulin resistance (HOMA-IR) and plasma glucose levels. Specific diet for the two groups was continued for another five weeks. Terminal blood samples were analyzed for plasma glucose, serum insulin, T3, T4 and TSH levels.

Results: Plasma glucose and serum insulin levels were found significantly elevated (p=0.001) in the diabetic group. HOMA-IR was increased in the diabetic group (p=0.001). Serum T4 and TSH levels were significantly elevated (p=0.001) while no significant change in serum T3 was noticed in the diabetic group. Pearson correlation coefficient revealed positive correlation between insulin resistance and T4 level (r=0.36) in the diabetic group. Positive correlation was found in the diabetic group between glucose level and T3 (r=0.36) and plasma glucose and T4 (r=0.35).

Conclusion: Development of thyroid dysfunction and insulin resistance interplay in development of hyperglycemia in type 2 diabetes mellitus.

Key Words: Type 2 diabetes mellitus, thyroid hormones, insulin resistance

Introduction

Little is known about the relationship between thyroid function and insulin resistance. With thyroid hormone concentration in normal range, even minor differences seem to be significant. Thyroid hormones have been documented to be associated with insulin resistance even in euthyroid range.1

Insulin resistance, a cardinal feature of Type 2 Diabetes Mellitus (T2DM), is characterized by impaired ability of insulin to inhibit hepatic glucose output and to stimulate glucose uptake.2 Patients with T2DM have been documented to have similar prevalence of hypothyroidism or hyperthyroidism as that in general population.3 Increased insulin resistance has been associated with higher circulating levels of insulin which leads to the increased thyroid proliferation.4

Insulin and thyroid hormones play an important role in glucose homeostasis.5 A complex mechanism involving, food intake, regulation of insulin secretion and its action on target tissues help in achieving glucose homeostasis. Action of thyroid hormones is an important determinant of glucose homeostasis.6 It has been studied that both triiodothyronine (T3) and insulin stimulate the expression of hexokinases and glycogen synthase which are respectively responsible for the uptake and disposal of glucose via the formation of glucose-6-phosphate and glucose-1-phosphate.7 Contrarily thyroid hormones oppose the action of insulin by stimulating gluconeogenesis and glycogenolysis, regulate the expression of genes such as GLUT-4 and phosphoglycerate kinase, involved in glucose transport and glycolysis respectively, and act synergistically with insulin in glucose uptake and disposal in peripheral tissues.8,9

Studies have shown that triiodothyronine (T3) stimulate GLUT4 messenger RNA and protein expression in skeletal muscle and elevates basal glucose uptake.10 Low normal serum free thyroxine (FT4) levels are significantly associated with high insulin resistance and subjects with low normal thyroid function have increased risk of cardiovascular diseases.11 Studies have suggested higher levels of total thyroxine (TT4) within euthyroid range in diabetic subjects as compared to the control group. Increased TT4 is related to abnormal glycemic levels and insulin resistance indicating the involvement of thyroid gland in insulin resistance.12 The present study
is designed to study thyroid hormone levels and insulin resistance in type 2 diabetic rats.

**Material and Methods**

The study was conducted on 60 healthy Sprague Dawley rats. Average weight of each rat was 250 ± 50 grams. Diabetic rats were excluded at the start of the study by estimating plasma glucose levels by glucometer. Rats with thyroid disease were excluded by measuring serum TSH levels. The rats were randomly divided into two groups, each having 30 rats. Rats in control group were fed on normal pellet diet and diabetic group was fed high fat diet and water ad libitum. After 2 weeks, an intraperitoneal injection of streptozotocin was administered to diabetic group and normal saline to the control group. HOMA-IR was calculated after 1 week to confirm the induction of T2DM by taking tail vein blood sample. Afterwards, rats were continued with the same diet for next 5 weeks.

At the end of study, rats were placed one by one in a closed glass chamber containing ether soaked cotton in the main laboratory of animal house for intracardiac sampling and blood was drawn with the help of 5.0 ml disposable syringe. After centrifugation, the plasma was pipetted out of the sodium fluoride tubes and put in the polypropylene storage tube. Plasma glucose was estimated by glucose oxidase method and serum insulin was done by ELISA technique. Serum T3, T4 and TSH levels were assayed by ELISA. The statistical significance of differences between the control and diabetic groups was determined by applying independent sample's t-test. p-value ≤ 0.05 was considered significant. Pearson correlation coefficient was worked out to find correlation between insulin resistance, thyroid profile and glucose levels.

**Results**

The blood glucose and TSH levels were checked at the beginning of the study which revealed that all the rats of the two groups were euthyroid (TSH=0.36 µU/ml) and euglycemic (glucose=94.24 mg/dl). Plasma glucose level more than 200mg/dl and HOMA-IR more than 2 in the diabetic group confirmed the development of T2DM (Table 1).

The comparison of plasma glucose, serum insulin, HOMA-IR and thyroid profile of the control and diabetic groups at the end of study period (8th week) revealed significant difference in plasma glucose levels between the two groups (p<0.001). Serum insulin levels were significantly raised in the diabetic group (p<0.001) (Table 2). HOMA-IR of the diabetic group (16.37±0.41) was significantly higher (p<0.001) as compared to the control group (0.335±0.24). There was no change in serum T3 levels in the diabetic group however serum T4 and TSH levels were found significantly raised (p<0.001) in the diabetic group as compared to the control. Pearson correlation coefficient between insulin resistance, thyroid profile and glucose levels in the control and diabetic groups showed strong positive correlation between HOMA-IR and T4 (r=0.36, p=0.04). Positive correlation was found between plasma glucose, T3 and T4 in the diabetic group (Table 3).
Discussion

The interplay of insulin and thyroid hormones in maintaining glucose homeostasis in type 2 diabetes mellitus was observed in the present study. The animal model closely resembling the metabolic characteristics of type 2 diabetic human was used in the study. The induction of T2DM was confirmed by the development of hyperglycemia (268.46 ± 5.09 mg/dl) and insulin resistance manifested by hyperinsulinemia (15.86 ± 0.338 µU/l) and raised HOMA-IR (8.878 ± 0.212).

The study was carried out for a period of 8 weeks after which the analysis of terminal blood samples revealed hyperglycemia (297.73±27.05 mg/dl), hyperinsulinemia (22.29±0.49 µU/l) and elevated HOMA-IR (16.37 ± 0.41) in the diabetic rats. In a study conducted by Srinivasan et al (2005), Sprague Dawley rats developed hyperglycemia which was similar to the finding in our study. A study on the magnitude of hyperglycemia and oxidative stress in experimental diabetic rats revealed elevated blood glucose levels after high fat diet (22% fat) and I/P injection of STZ in the dose of 10mg/kg body weight daily.16

Induction of T2DM and insulin resistance in Sprague Dawley rats, by feeding high fructose diet (60% fructose) for 4 weeks, without administration of STZ, led to raised insulin levels were raised.17 The rise in insulin level in present study was much higher. It can be ascribed to the prolonged duration (8 weeks) for which high fat diet was fed to the diabetic rats even after the development of T2DM. In an animal model of T2DM two groups of male Sprague Dawley rats were studied. One group was fed normal chow and the other group was fed high fat diet for a period of two weeks. Analysis of blood samples of both the groups revealed increased level of serum insulin in fat fed STZ rats as compared to chow fed STZ rats.18 This finding revealed that high fat diet contributed to the induction of insulin resistance which resulted in raised level of serum insulin.

Raced HOMA-IR (16.37 ± 0.41) in the diabetic rats of present study indicated the presence of insulin resistance which was comparable to the taurence supplemented type 2 diabetic OLETF rats. At the end of 12 weeks of the study it was found that HOMA-IR was high in diabetic rats.19 The effect of fish oil and olive oil on insulin resistance had been studied in male Wistar rats. It was observed that in rats fed on high fat-butter diet developed significantly higher (p<0.05) insulin resistance.20 Male Sprague Dawley rats were given high fat diet containing 41.2 % fat and a single intraperitoneal injection of STZ (40mg/kg). Sampling done after a period of 8 weeks revealed an elevated HOMA-IR in the diabetic rats, comparable to the HOMA-IR of diabetic group of our study.21 As insulin is known to have a stimulatory effect on proliferation of thyrocytes, low levels of insulin can lead to decreased level of thyroid hormones specially T4 in their study.22,23

Screening of diabetic subjects for thyroid dysfunction revealed that 17% patients had high total T4 while fT4 and total T3 and fT3 levels did not differ much. 28% diabetic patients had low plasma thyroid hormones level.24 These findings could be due to the reason that the subjects in their study were on oral hypoglycemics and insulin therapy. Phenylthioureas are known to decrease the levels of fT4 and TT4 while exogenous insulin administration increases the level of fT4 and decreases T3 by inhibiting the hepatic conversion of T4 into T3.25 Glycemic status is known to alter TRH and TSH levels, therefore the presence of raised and low levels of thyroid hormones in different patients of their study, reflected the glycemic status of these diabetic patients.26

High levels of circulating insulin in type 2 diabetes results in thyroid gland proliferation and formation of thyroid nodules. Insulin and insulin like growth factor (IGF1) have been known to modulate the regulation of thyroid gene expression and is considered an important factor in thyrocyte proliferation and differentiation.27 A study conducted to assess the association between insulin resistance and thyroid hormones in subjects having subclinical thyrotoxicosis revealed T4 and free T4 levels on the higher side of the normal range.28 –30

Conclusion

Glucose levels and insulin resistance is positively associated with thyroid hormone levels in STZ induced T2DM.

References


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