CHAPTER - I

Study of glucose regulation by pancreas under experimentally induced hypo and hyperthyroid conditions in Rat.
INTRODUCTION

Thyroid hormone is a metabolic hormone so it is very important for glucose homeostasis. Impaired glucose tolerance is a frequent complication of hyperthyroidism. This alteration changes both insulin secretion and degradation in humans (Dimitriadis et al., 1985). Impairment in the insulin-induced suppression of glucose production in hyperthyroid patients has been reported (Dimitriadis et al., 1985; Laville et al., 1984). Despite immense investigations, the role of thyroid gland in glucose homeostasis has remained elusive. Therefore, investigation is needed to uncover this complex interaction between thyroid hormones and glucose homeostasis in experimentally induced hypothyroid and hyperthyroid rat as mammalian models. Hyperthyroidism has been recognized as a reversible cause of hyperglycemia for many decades (Dimitriadis and Raptis, 2001). Several clinical investigators have clearly shown that hyperthyroidism, both spontaneous and experimental, results in an unopposed activation of gluconeogenesis (Kreines et al., 1965). This finding has been attributed to the excessive availability of substrate (alanine) resulting from the thyrotoxicosis-associated catabolic state (Singh and Snyder, 1978). Although thyroid hormone excess has been consistently found to induce insulin
Thyroid dysfunction status with special reference to polymorphism of thyroid peroxidase (TPO) gene resistance (Dimitriadis and Raptis, 2001), there are only a few studies examining insulin action in thyroid hormone deprivation in humans, with conflicting results.

Glucose regulation by insulin depends on the suppression of endogenous glucose production and the stimulation of peripheral glucose disposal. Hepatic glucose production is decreased (Okajima and Ui, 1979) or unchanged (Muller et al., 1988) in hypothyroidism, but there is little information on the effects of insulin in peripheral tissues and in particular, in vivo.

Whole-body sensitivity of glucose disposal to insulin in hypothyroid patients has been examined by euglycemic-hyperinsulinemic clamps (Harris et al., 1993) and intravenous (IV) or oral administration of glucose (Lamberg, 1965; Andreani et al., 1970; Jackson et al., 1970) and found to be normal, increased or decreased (Dimitriadis et al., 2006). In the forearm muscles of hypothyroid patients, the sensitivity of glucose disposal to insulin is found to be normal, whereas in adipocytes isolated from hypothyroid patients and
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that of glucose transport was decreased (Pedersen et al., 1988). In rats made hypothyroid, the responsiveness of glucose disposal to insulin was decreased in muscle (Dimitriadis et al., 1989; Dimitriadis et al., 1997; Cettour-Rose et al., 2005) but increased in adipocytes (Correze et al., 1979).

Therefore, investigation is needed to uncover that complex interaction between thyroid hormones and glucose homeostasis in experimentally induced hypo and hyperthyroid rat.

This chapter briefly addresses the role of thyroid hormone action on glucose homeostasis with a specific focus on the significance of the peripheral metabolism of thyroid hormone in the regulation of glucose homeostasis and insulin sensitivity.

MATERIALS AND METHODS

The laboratory experiment carried out in the common rat (Rattus rattus) as a mammalian model. Methimazole (Sigma, USA) was used for the induction of hypothyroidism and Eltroxin, Thyroxine Sodium tablets (Glaxo, India) for hyperthyroidism. Normal young adult rat aged 8-10 weeks and weight 80-85
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g were housed in polypropylene cages and were acclimatized in laboratory condition for a week with natural light and dark schedules prior to experimentation. The animals were fed standard rodent diet and water. For experimental induction of hypothyroid and hyperthyroid animals treated with Methimazole 20 mg/kg body weight/day and Eltroxin 600 µg/kg body weight/day for 14 days respectively.

**Oral Glucose Tolerance Test (OGTT)**

For oral glucose tolerance test (OGTT) both the control and treated (hypo and hyperthyroid) rats after 18 h of fasting followed by challenge with glucose (25 mg glucose/100 g body weight) and at the following time point after oral glucose infusion: 0.5, 2.5 and 24 h blood was collected from the tail veins of control and experimental rats. Blood glucose was estimated using a blood glucose monitoring system (ACON Laboratories, Inc. San Diego, USA).

**Assay of T₃ and T₄ hormones**

Serum T₃ and T₄ levels were determined by radioimmunoassay (RIA). Blood was drawn directly from heart for serum T₃, T₄ hormones. T₃ and T₄ concentration was determined by radioimmunoassay (RIA). ¹²⁵I-labeled
thyroid hormone (either T₃ or T₄) competes with thyroid hormone in the serum sample and for antibody sites on the tube, in the presence of blocking agents for thyroid hormone binding proteins.

**Histology**

Tissue samples from pancreas of both experimental and control animals were placed in Bouin’s fixatives (Parakkal, 1961) for overnight. Tissues were dehydrated through graded alcohols, embedded in paraffin wax, sectioned (5μm thick) and stained with hematoxylin-eosin. The stained sections were analyzed for detailed cellular changes under the light microscope.

**Hematoxylin eosin staining Procedure:**

1) The fixed tissues were transferred to 70% alcohol for 18 h and carried through graded alcohols for dehydration.

2) After dehydration all the tissues were kept in cedar wood oil for some days and subsequently transferred to xylene for 10 minutes prior to infiltration in paraffin.
3) Tissues were then transferred to half xylene and half paraffin mixture and then to full paraffin (52°-53°C melting point).

4) 5μm thick sections were then made and mounted on glass slides.

5) The slides were carried through xylene, graded ethanol and water.

6) After hydration the slides rinsed into Hematoxylin solution.

7) Washed briefly in tap water.

8) Dehydrated by 70% and 90% ethanol.

9) Then stained with eosin.

10) Washed by 90% ethanol.

11) Dehydrated in absolute ethanol.

12) Cleared in xylene and mounted in D.P.X.

Hematoxylin stains DNA and Eosin stains protein.

\[ \text{Nuclei} \quad \text{-------------------} \quad \text{blue} \]

\[ \text{Cytoplasm} \quad \text{--------------} \quad \text{pink to red} \]

**Liver Glycogen**

Liver glycogen was determined by the colorimetric method (Kemp & Van Heijningen, 1954) by digesting 1 g fresh rat liver in 30% KOH and treatment
Thyroid dysfunction status with special reference to polymorphism of thyroid peroxidase (TPO) gene with the anthrone reagent. Finally glycogen contents were measured by colorimeter in 620 nm wavelength (Chakrabarti et al.; 2007).

RESULTS

T₃ and T₄

The results showed significant changes in T₃, T₄ hormones (Fig. 4 A & B). The serum T₃ and T₄ level was significantly reduced in hypothyroid rat whereas in contrast the level significantly increased in hyperthyroid rat as expected and confirming that they were indeed in a hypo and hyperthyroid state.

<table>
<thead>
<tr>
<th>Rats</th>
<th>T₃ (ng/dl)</th>
<th>T₄ (mcg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>43.67 ± 0.44</td>
<td>4.36 ± 0.24</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>27.56 ± 0.26**</td>
<td>1.83 ± 0.23*</td>
</tr>
<tr>
<td>Hyperthyroid</td>
<td>63.88 ± 0.57**</td>
<td>6.80 ± 0.26*</td>
</tr>
</tbody>
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Fig. 4: A&B- Plasma concentrations (ng/dl) of T₃ (A) and T₄ (B) in normal, hypothyroid and hyperthyroid rat. Values are expressed as mean ± SE from 6 rats. P values :*< 0.001; **<0.01

Liver glycogen level

Liver glycogen increased dramatically in both hypothyroid (by ~22.8-fold) and hyperthyroid (by ~10.45-fold) rats (Fig. 5).
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Table 2: Liver glycogen content (mg/g wet tissue) in hypothyroid and hyperthyroid rats. Values are expressed as mean ± SE from 6 rats. P values: *< 0.001

<table>
<thead>
<tr>
<th>Normal</th>
<th>Hypothyroid</th>
<th>Hyperthyroid</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.27±0.31</td>
<td>74.74±0.47*</td>
<td>34.20±0.62*</td>
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**Oral Glucose Tolerance Test (OGTT)**

While hypothyroid rats displayed low glucose level (by 18%) no change in glucose level was seen for hyperthyroid rats (Fig. 6). In control rats the blood glucose level returned to the normal level after 2.5 h of glucose feeding. Like control rats, in hypothyroid rats glucose level increased by
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90% after 0.5 h of glucose challenge but the elevated glucose did not return to basal level even after 24 h of glucose challenge (Fig. 6). Unlike control and hypothyroid rats the increments of glucose level after 0.5 h of glucose challenge was modest (by -22%) in hyperthyroid rats. However, like hypothyroid rats, the glucose remained elevated even after 24 h of glucose feeding.

<table>
<thead>
<tr>
<th>Rats</th>
<th>0 h</th>
<th>1.5 h</th>
<th>2.5 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>40.30±1.60</td>
<td>71.10±1.85</td>
<td>39.60±1.80</td>
<td>37.80±1.87</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>33.00±1.66</td>
<td>62.60±2.23</td>
<td>52.30±2.19</td>
<td>41.80±1.94</td>
</tr>
<tr>
<td>Hyperthyroid</td>
<td>37.80±2.04</td>
<td>46.30±2.50</td>
<td>49.80±1.19</td>
<td>47.80±1.94</td>
</tr>
</tbody>
</table>
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Fig. 6: Blood glucose level in response to glucose tolerance test in control, hypothyroid and hyperthyroid rats. Values are expressed as mean ±SE from 6 rats. *P < 0.001; **P < 0.01

Histology of Pancreas

Hyperthyroid rats exhibited marked changes in the general cytomorphology of islets of Langerhans (Fig. 7) as evidenced by the enlargement and disruption of islets and disorientation of the cells. The cells lost the individual boundary. Small numbers of islets cells were found to be picnotic.
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Fig. 7: Histology of normal, hypothyroid and hyperthyroid rat pancreas.

In the picture I mentioned the total magnification

Total magnification = magnification of eyepiece x magnification of objective lens
DISCUSSION

Rats were induced experimentally to be hypo and hyperthyroid. The results showed significant changes in T₃, T₄ hormones (Fig. 4 A&B) and histology of pancreas (Fig. 7). The serum T₃ and T₄ level was significantly reduced in hypothyroid rat whereas in contrast the level significantly increased in hyperthyroid rat as expected and confirming that they were indeed in a hypo and hyperthyroid state.

Hypothyroidism is associated with decreased gluconeogenesis and glycogenolysis resulting in increment in glycogen in liver. In addition, hypothyroidism lowers the glycogen phosphorylase activity in the liver (Storm et al., 1984). Consistent with these findings profound increments in liver glycogen in hypothyroid rats were observed. In contrast, the hyperthyroid state is associated with low hepatic glycogen levels, but paradoxically with a high activity of glycogen synthase and low activity of glycogen phosphorylase. In hyperthyroidism, insulin-stimulated rates of glucose utilization in muscle to form lactate are increased mainly because of a decrease in glycogen synthesis. In the present study, elevated glycogen content in the liver of hyperthyroid rats is paradoxical and may be due to the
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high activity of glycogen synthase (Betley et al., 1993). In hyperthyroid humans as well as in experimental thyrotoxicosis in animals, glucose turnover and hepatic glucose production are increased due to increased metabolic rate and peripheral glucose utilization (Menahan and Wieland, 1969). Experimental as well as spontaneous hyperthyroidism in humans cause increased glucose production and impaired suppression of glucose production by insulin (Moreno et al., 1997; Shen et al., 1988).

Consistent with these previous reports increments in blood glucose levels were observed after OGTT and the hyperglycemia persisted even 24 h after glucose load. It is believed that insulin response to a glucose load is relatively decreased in hyperthyroidism and that an inability to increase their insulin response further impairs glucose tolerance in low β-cell responder (Ikeda et al., 1990; Kabadi and Eisenstein, 1980). It is possible that T₄ causes deleterious effects on β-cell function thereby impairing insulin secretion after an oral glucose load. In line with this thought, Lenzen (1978) reported that injections of T₄ (50-2000 μg/kg/day for 5 days) dose-dependently inhibited glucose-induced insulin secretion in the isolated pancreas. It has also been reported that hyperthyroid patients with younger age (<30 years) showed an increased secretion of insulin to compensate
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hyperglycemia after glucose load, but older patients (>31 years), who may have a low β-cell reserve, showed a blunted response of insulin (Komiya et al., 1985). In addition, insulin sensitivity is altered in hyperthyroid patients (Ohguni et al., 1995; Gonzalo et al., 1996). In the present study, the reason behind impaired glucose tolerance in response to glucose overload in hypothyroid rats is not known and further experiments needs to be performed.

Hyperthyroid rats exhibited marked changes in the general cytomorphology of islets of Langerhans. It has been revealed that thyroxine decreased secretory response of beta-cells to glucose when intravenous glucose tolerance test was carried out and to be lesser degree when pectoral glucose tolerance test was realized. Insulin level in the peripheral blood plasma at various times after glucose injection was significantly lower than in control animals (Zhurova and Poltorak, 1980). Hyperthyroidism impairs glucose-stimulated insulin secretion (GSIS) and insulin action (Holness et al., 2005). The reason for the reduction of the β-cell volume of the pancreas after thyroxine treatment is apparently an increased rate of β-cell apoptosis. In parallel, thyroxine treatment increased the rate of apoptosis in rat pancreatic ductal cells which are considered to contribute to the pool of stem cells from
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which insulin producing beta cells originate. An increased rate of β-cell death due to apoptosis causes a decrease of insulin content and glucose-induced insulin secretion from the pancreas in hyperthyroidism (Jorns et al.; 2002). The resulting reduction of β cells in the pancreas can provide an explanation for the decrease of glucose tolerance in hyperthyroidism.

Thyroid hormones may act as insulin antagonists. Hyperthyroidism induces glucose intolerance in animals and humans. In prone individuals, hyperthyroidism can induce a so-called ‘thyroid diabetes’. Thyroxine treatment causes a state of experimental hyperthyroidism and reduces the pancreatic insulin content and glucose induced insulin secretion. This is accompanied by a reduction in the β-cell volume of the pancreas.

The increased loss of β cells from the endocrine pancreas due to thyroid hormone induced apoptosis or necrosis or cell loss in the hyperthyroid state puts an increased demand on β cell for insulin.

Thus medical efforts should be directed towards prevention of over-challenging the insulin secretory reserve of the endocrine pancreas such as in hyperthyroidism or during exogenous thyroid hormone administration. This
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could be particularly important in people with a limited endogenous insulin secretory capacity who are most susceptible to the development of ‘thyroid diabetes’ such as elderly patients with a borderline insulin secretory capacity. This should be kept in mind also when, for therapeutic reasons, thyroid hormone supplementation is considered.