CHAPTER II

REVIEW OF LITERATURE
CHAPTER II

REVIEW OF LITERATURE

The simultaneous occurrence of DM with various hormonal diseases (e.g. pituitary, adrenal or thyroid diseases) is frequently observed. However, a significant number of endocrine disorders are associated with varying degrees of glucose intolerance. Indeed, a sustained excess of hormones, antagonistic to insulin (e.g. glucocorticoids, catecholamines, glucagon), or interfering with insulin secretion (e.g. catecholamines, hypokalemia) is often associated. Therefore, acromegaly, Cushing syndrome, primary hyperaldosteronism, hyperthyroidism, glucagonoma and other neuro endocrine tumors (NET) are included in endocrine associated diabetes. Although retinal, renal, and neurological complications are uncommon in patients with endocrine associated diabetes.\(^{(72)}\) Data on the prevalence of DM vary according to the definition of the disease. Based on increased fasting glucose levels, as in National Health and Nutrition Examination Survey (NHANES-III), approximately 14% of all adults suffer from either DM or an impaired fasting glucose level with a substantial proportion of subjects being unaware of their disease. The total prevalence of DM is increasing and is projected to rise to 366 million worldwide in 2030, affecting 4.4% of all age groups.\(^{(73, 74)}\)

DM and thyroid dysfunction are the two most common endocrine disorders, and appear to be closely linked. Among human autoimmune conditions, the strongest association is seen between two.\(^{(75)}\) Thyroid disorders are most frequently induced by an autoimmune process. Data on the global prevalence are again subject to considerable variations because of different definitions of the conditions, particularly of subclinical thyroid dysfunction. In population studies subclinical hypothyroidism is significantly higher in
Defects in carbohydrate metabolizing machinery and consistent efforts of the physiological system to correct the imbalance in carbohydrate metabolism, place an overexertion on the endocrine system. Continuing hyperglycemia exacerbates the metabolic disturbances and leads primarily to deterioration of endocrine control. The major alterations in thyroid hormone system are a reduction in the TSH stimulation of the thyroid gland, probably caused by central hypothyroidism, and in the peripheral generation of T₃ from T₄. There are also important structural changes in the thyroid gland and pituitary that are accompanied by marked alterations in their secretory activity. In addition, T₄ de-iodination to T₃ in peripheral tissues is decreased. The physiological and biochemical interrelationship between insulin and the influence of both insulin and iodothyronines on the metabolism of carbohydrates, proteins, and lipids are seen.

Diabetes has also been associated with numerous thrombotic, atherosclerotic, and CVD. DM is one of the most common human metabolic diseases, and the derangements in lipid metabolism in this are often important determinants of course and status of the disease. The epidemic of CVD especially coronary heart disease is emerging in rural India and accelerating in urban India. CVD mortality in India accounted for 16.9% of all CVD deaths worldwide. Patients with DM are at risk for premature atherosclerosis even with normal plasma lipid levels. Studies show the lipid metabolism is influenced by several hormones such as insulin, growth hormone, thyroid hormone.

The abnormally high concentration of serum lipids in diabetes is mainly a result of the increase in mobilization of free fatty acids from peripheral depots. This happens because reduced insulin levels increase the activity of the hormone sensitive lipase. On the other hand, glucagons, catecholamines, and other hormones enhance lipolysis. The marked hyperlipidemia that characterizes the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on fat depots. The
increase and fall in the individual lipoprotein levels is a reflection of TC levels; that is, the levels of VLDL-C, LDL-C, and HDL-C increase or decrease with the level of TC, and it is their ratio that determines the pathophysiology of lipoprotein metabolism. Studies observed a higher concentration of Tg’s in hyperlipidemic and diabetic hyperlipidemic patients. These increased levels may due to increased secretion of VLDL by the liver, disturbed catabolism of VLDL and decreased removal of Tg’s due to diminished lipoprotein lipase activity. Increased cholesterol in hyperlipidemic patients with and without diabetes may be due to increase in biosynthesis or diminished clearance from the blood. Plasma cholesterol levels are often elevated in hypothyroidism, which may be associated with normal or increased Tg’s. Conversely, hyperthyroid patients have reduced plasma cholesterol, also associated with elevated or normal plasma Tg’s.

2.1 Prevalence of Thyroid Disorders in General Population and Diabetic Patients

The prevalence of thyroid disorder in diabetic population was reported to be 13.4% with higher prevalence (31.4%) in female T2DM patients as compared to (6.9%) in male T2DM patients. The prevalence of thyroid dysfunction in T2DM patients was reported to be 12.3% in Greece and 16% in Saudi Arabia by Akbar et al. Considerably, T2DM patients were more prone to thyroid disorders. Both hyperthyroidism and hypothyroidism are graded phenomena, ranging from very mild cases in which biochemical abnormalities are present without any symptoms or signs of thyroid hormone excess or deficiency, to very severe cases that may end up as a life-threatening thyrotoxicosis crisis or myxoedema coma. Their prevalence varies according to the studied population. The Whickham survey, conducted in the north of England, revealed a prevalence of overt thyrotoxicosis or hypothyroidism of at least 2% in females and 0.2% in males. Subclinical hypothyroidism, the most prevalent form of thyroid diseases, is more common in
females and in the elderly, reaching a prevalence of up to 20% in women over 60 years old.\(^{(92)}\) The incidence of progression to overt thyrotoxicosis is approximately 5% per year; and patients with autonomous thyroid adenoma or nodular goitre are especially at risk.\(^{(93)}\) The main causes of hypothyroidism and hyperthyroidism are Hashimoto’s thyroiditis and Graves’ disease respectively, both of an autoimmune nature. Prevalence studies show that Autoimmune Thyroid Disease (AITD) is higher in type 1 diabetes. Perros et al. reported thyroid dysfunction in up to 31.4% of adult type 1 diabetic females. Moreover, in children with type 1 diabetes, the proportion of positive thyroid antibodies might increase up to 20% and about 3–8% of children and adolescents with type 1 diabetes have been reported to develop autoimmune hypothyroidism. Postpartum thyroiditis, a rather common event, with an incidence of 4–6% as evident from several population-based studies, is threefold higher (up to 25%) in women with type 1 diabetes.\(^{(94,95)}\) Although thyroid disease, overt or sub-clinical, is reported to be relatively common in type 1 diabetes, a longitudinal Australian study in type 2 diabetic women without known thyroid disease showed that sub-clinical hypothyroidism is a common, but incidental finding.\(^{(18)}\) In view of the relatively high prevalence of both endocrinopathies, it is important to investigate all diabetic patients for thyroid disorders. However, screening has been recommended only in children and adolescents with type 1 diabetes.\(^{(96)}\) TSH should be tested several weeks after the diagnosis of type 1 diabetes, when metabolic control has been established. If the TSH level is normal, patients should have a repeat measurement every 1–2 years. Additional thyroid function testing should be obtained whenever thyroid dysfunction is suspected or thyromegaly is detected.\(^{(97,98)}\)

With regards to diabetic adults, there is no consensus as to whether screening for thyroid disorders should be mandatory.
2.2 Genetics

Epidemiological evidence suggests a common genetic background for both thyroid disease and DM. Autoimmunity is likely to be the result of particular environmental factors on genetically susceptible individuals, causing loss of self-tolerance and thereby triggering disease.\(^{(99)}\) Both organ specific T-cell-mediated diseases, share a strong genetic susceptibility as they frequently occur in the same individuals and in the same families. Thus, genetic–epigenetic interaction is likely to play a pivotal role in the shared genetic predisposition to endocrinopathies.\(^{(100, 101)}\) Autoimmune causes are reported to be responsible for the genetic dysfunction in the diabetic patient suffering from thyroid related disorders. However, these findings advocate an immense clinical evidence to support association between T1DM (Type 1 diabetes mellitus) and AITD.\(^{(99, 106)}\) Arrays of genes involved in metabolism of glucose are modulated by active thyroid hormone T3 by binding to the thyroid hormone receptors. These receptors are derived from TR\(\alpha\)1, TR\(\beta\)1, TR\(\beta\)2, and TR\(\beta\)3. These are four major T3 binding isoforms.\(^{(102)}\) TR\(\alpha\)1 is hypothesized to regulate the metabolic effects of thyroid hormone. TR\(\beta\)1 and TR\(\beta\)2 are related with maintenance of hypothalamic-pituitary-thyroid axis and keeping the euthyroid state.\(^{(103)}\)

Certain human leukokocyte antigens (HLA), encoded within the major histocompatibility complex (MHC) have been recognized for more than 30 years as being over represented in both patient groups. HLA class II presents peptide antigens to T cells, and HLA DR3 allele between AITD and T1DM contribute to the joint genetic susceptibility.\(^{(104, 105)}\) A single specific HLA class II pocket amino acid signature confers joint susceptibility in the same individual by inducing significant structural changes in the MHC II peptide binding pocket.\(^{(106)}\) Apart from the MHC locus, an increasing number of other genes have recently been suggested to be associated with an increased
risk for both conditions. Potential genetic susceptibility loci have been identified at the following loci: CTLA4 (on chromosome 2q33), PTPN22 (1p13), IFIH1 (2q24), CD25 (10p15), C12orf30 (12q24), ERBB3 (12q13), PTPN2 (18p11), KIAA0350 (16p13), CD226 (18q22), INS (11p15), FCRL3 (1q23) and the TSH receptor (14q31).\(^{(107)}\) An extensive study in families with a high frequency of T1DM and AITD revealed that CTLAA-4 carries a major genetic risk for the joint diagnosis of T1DM and AITD.\(^{(108)}\)

There are few studies suggesting a direct genetic basis of thyroid disease associated with T2DM. Recent data on polymorphism of the deiodinase type 2 (DIO2) gene, Thr92Ala, suggest that homozygosity for this polymorphism is associated with an increased risk of T2DM.\(^{(109)}\) These data were supported by a meta analysis in almost 11,000 individuals and indicate a possible role of intracellular T3 on insulin sensitivity.\(^{(110)}\) Positive regulation of insulin sensitive GLUT-4 transcription\(^{(111)}\) showed that there were profound genomic effects of T3 on hepatic glucose metabolism. TR expressed in the hepatocyte and stimulation of T3 sensitive neurons in the hypothalamus-modulated hepatic glucose production via sympathetic projections to the liver are mediated by circulating gluco-regulatory hormones.\(^{(111)}\) Recent findings have elucidated polymorphism of DIO2 gene, Thr92Ala, which suggest homozygosity for this polymorphism which in turn is responsible for enhanced risk of T2DM.\(^{(109)}\)

### 2.2.1 Effect of thyroid hormones on the liver

Various genes have been identified which are identified with gluconeogenesis, glycogen metabolism, and insulin signaling. These include glucose 6 phosphate, protein kinase B, \(\beta2\) adrenergic receptor, inhibitory G protein, phosphoenolpyruvate kinase (PEPK)\(^{(112)}\), pyruvate carboxylase (PC), GLUT 2\(^{(113)}\), malic enzyme\(^{(114)}\), and carbohydrate response element binding protein (ChREBP).\(^{(66)}\) A raised hepatic expression of GLUT 2 in hyperthyroid rats was observed as compared to hypothyroid rats.\(^{(113)}\)
Investigations using skeletal muscles in hypothyroid and euthyroid humans have revealed a discernable influence on the downregulated expression of GLUT-5 but not GLUT-4.\textsuperscript{135,146} Glucose oxidation and glycogen synthesis are reduced in hypothyroidism.\textsuperscript{70} Simultaneous increase in the insulin sensitivity occurs when the levels of thyroid hormone were increased. This phenomenon is governed by intracellular generation of T3 as polymorphisms of DIO2 with reduced T3 generation and also contributes to insulin resistance.\textsuperscript{109} In hyperthyroidism, the expression of GLUT2 is increased as compared to euthyroid state.\textsuperscript{116} In such conditions, perturbations in lipid metabolism further link TH to insulin resistance.\textsuperscript{116}

\subsection{Effect of thyroid hormones on the skeletal muscle}

The various genes which influence the interaction of thyroid hormone and skeletal muscles include GLUT1, GLUT4 \textsuperscript{113}, β2 adrenergic receptors \textsuperscript{101}, phosphoglycerate kinase (PGK) \textsuperscript{105}, PPAR gamma coactivator-1 alpha (PGC-1 alpha) \textsuperscript{117}, and mitochondrial uncoupling protein (UCP).\textsuperscript{118} Amongst the various genes identified, GLUT-4 and UCP-3 have been studied in detail. In the skeletal muscles, GLUT 4 has been proven to be mediated by the influence of T3, and it can elevate basal and insulin mediated transport of glucose.\textsuperscript{113}

\subsection{Pathological Mechanisms Common to Thyroid Disorders and Diabetes}

Thyroid hormones exert profound effects in the regulation of glucose homeostasis. These effects include modifications of circulating insulin levels and counter-regulatory hormones, intestinal absorption, hepatic production and peripheral tissues uptake of glucose. Thyroid hormones oppose the action of insulin and stimulate hepatic gluconeogenesis and glycogenolysis.\textsuperscript{119,120} They up-regulate the expression of genes
such as GLUT-4 and phosphoglycerate kinase, involved in glucose transport and glycolysis respectively, thus acting synergistically with insulin\textsuperscript{(101, 121)} in facilitating glucose disposal and utilization in peripheral tissues. Thyrotoxic patients show an increased glucose turnover with increased glucose absorption through the gastrointestinal tract, postabsorptive hyperglycaemia and elevated hepatic glucose output, along with elevated fasting or postprandial insulin and proinsulin levels, elevated free fatty acid concentrations and elevated peripheral glucose transport and utilization.\textsuperscript{(122,34)} Thyrotoxicosis has been associated with either normal, decreased or increased β-cell function.\textsuperscript{(123)} However, it has been suggested that proinsulin in excess may account for the hyperinsulinemia observed with higher release of insulin both after absorption and at baseline, when compared with the euthyroid situation or with control subjects. Moreover, recent studies have shown that thyroid hormones increase β-cell apoptosis and that this could be one major element responsible for deterioration of glucose tolerance in thyrotoxicosis.\textsuperscript{(124, 125)} T2DM patients with thyroid dysfunction have been proven to be more susceptible to ketosis\textsuperscript{(126)} and ketogenesis.\textsuperscript{(127)} There is marked increase in the skeletal glucose utilization in hyperthyroid state.\textsuperscript{(128)} Increased glucose utilization has been reported to be mediated by insulin stimulated glucose oxidation rates.\textsuperscript{(129, 130)} Under such conditions, reduced glycogenesis has been reported due to insulin stimulated non oxidative glucose disposal, which is accompanied by redirection of intracellular glucose towards glycolysis and lactate formation.\textsuperscript{(151)} The transport of lactate from periphery to liver leads to enhanced production of glucose via Cori’s cycle. Hyperthyroidism has also been associated with enhanced insulin sensitivity.\textsuperscript{(131)} Increased peripheral insulin resistance has been coupled with elevated expression of bioactive inflammatory mediators including adipokines (IL-6 and TNF-alpha) \textsuperscript{(116)}which lead to insulin resistance.
In hypothyroidism, glucose homeostasis is also affected although its clinical impact is less obvious. Decreased glucose disposal (as compared with euthyroid subjects) has been proved in hypothyroid patients by different methods including clamp studies\(^{(132, 133)}\), the arteriovenous difference technique in the anterior abdominal subcutaneous adipose tissue and forearm muscles after the consumption of a mixed meal, the insulin tolerance test and following intravenous or oral administration of glucose. Nonetheless, hypothyroidism results in unimpaired or decreased liver glucose output thereby compensating for insulin resistance present in peripheral tissues and accounting for the diminished insulin requirement for glycemic control in hypothyroid diabetic patients.\(^{(134, 135, 136)}\) As regards to β-cell function, normal or reduced basal plasma insulin levels have been described in hypothyroidism. These findings are quite consistent with the idea of attenuated endogenous glucose production in the hypothyroid state. Insulin resistance has been also reported in subclinical hypothyroidism, adding one more possible mechanism to the association of sub-clinical hypothyroidism and cardiovascular risk.\(^{(135)}\) The interaction between insulin resistance and lower thyroid function might be a key determinant for a more atherogenic lipid profile in these populations. Even though thyroid status, as assessed by plasma hormone levels, is a key indicator of glucose homeostasis, T\(_3\) intracellular pathways are also relevant. The hormonal message is modulated at a local level by a series of control steps, including the intracellular concentration of T\(_3\) via deiodinases, and the relative concentration of T\(_3\) receptor isoforms, co-activators, and co-repressors.\(^{(137, 138)}\)

2.4 Central Interactions of Thyroid Hormones on Glucose and Lipid Regulation

Recent data suggest an important role for hypothalamic regulation of glucose and lipid homeostasis. This was expected from human data showing defects in counter-regulation
of glucagon and the sympathetic nervous system in patients with hypothalamic vs.
pituitary defects, indicating an important role of hypothalamic glucose sensing.
Adenosine monophosphate kinase (AMPK), regulates cellular metabolism, integrates
nutritional and hormonal signals in the hypothalamus. Selective knock out (KO) of AMPK in Pro-opiomelanocortin (POMC) related neurons induces distinct alterations in energy homeostasis.\(^{(139, 140)}\) Inhibition of hypothalamic AMPK decreases peripheral glucose production. AMPK further links glucose regulation to FA synthesis via the carboxylation of acetyl-CoA to form malonyl-CoA, which is catalysed by ACC. Phosphorylation/inhibition by AMPK controls ACC, the rate-limiting step in the generation of malonyl-CoA, which can undergo reductive chain elongation to form C16/C18 long-chain FA catalysed by Fatty acid synthase (FAS).\(^{(141)}\) In parallel, malonyl-CoA suppresses FA oxidation by acting on the translocation of FA into mitochondria, which is catalysed by carnitine palmitoyl transferase-1 (CPT1). Targeting this pathway in diet induced obesity by inhibiting hypothalamic lipid oxidation is sufficient to normalize food intake and glucose homeostasis.\(^{(142)}\) THs directly affect these regulatory steps. In animals with selective liver sympathetic and parasympathetic denervation, hypothalamic T3 directly controls endogenous glucose production, whereas peripheral concentrations of the hormone, of insulin, glucagon or corticosterone have no effects. Peripherally, AMPK is dose and time dependently stimulated by T3.\(^{(143)}\) Studies in hypothyroid animals showed a selective increase in AMPKa1 concentrations and activity in the hypothalamus but not in muscle or white fat. AMPK activity decreased when T3 is chronically infused intracerebro-ventricularly in a dose not capable of increasing peripheral T3 levels. Further proof was added when AMPKa was specifically inhibited by stereotoxic delivery of a dominant- negative form of the gene into the ventromedial hypothalamus in euthyroid rats. The animals showed a clear phenotype
with weight loss, which was independent from food intake. Expression of energy-regulating neuropeptides in the hypothalamus was stable, but b-adrenergic drive to the peripheral brown adipose tissue was significantly increased. AMPK changes were linked to CPT1, and this may link TH-dependent regulation with peripheral energy-regulating peptides like ghrelin that also targets hypothalamic AMPK/CPT1.\(^{(139)}\) This is particularly important for ghrelin effects on AMPK/CPT1 in the counter regulation of hypoglycaemia but may also have long-term effects on energy homeostasis.

### 2.5 Clinical Aspects

THs are positively associated with insulin resistance not only in clinically diagnosed DM but also in subjects with a normal glucose tolerance. Indices of insulin resistance as judged by the Homeostasis Model Assessment (HOMA) (which assesses fasting and postprandial insulin resistance) are closely linked to TH status even in euthyroid, eumetabolic subjects, where HOMA is related to the increase in TH concentrations even within the normal range.\(^{(144)}\)

#### 2.5.1 Thyrotoxicosis

Excess THs lead to hyperglycaemia, via increased glucose absorption by the gastrointestinal tract, increased hepatic glucose output, hyperproinsulinaemia and hyperinsulinaemia, high free FA levels and increased peripheral glucose transport and metabolism. The increased hepatic glucose output constitutes a major element in the induction of hyperinsulinaemia, induction of glucose intolerance and the development of peripheral insulin resistance. Increased hepatic glucose output together with increased glycogenolysis in thyrotoxicosis constitutes major elements in the decrease in glucose tolerance.\(^{(145,146)}\) Despite increasing insulin clearance, hyperinsulinaemia is induced and peripheral insulin resistance develops. This is reflected in clinical observations in which hyperthyroidism precipitates subclinical diabetes and worsens glycaemic control in pre-
existing T2DM. The development of thyrotoxicosis may further induce diabetic ketoacidosis, a life-threatening condition. This is not restricted to T1DM but has also been described in patients with T2DM where ketoacidosis is provoked by the pronounced lipolytic effects and the increased hepatic β-oxidation of thyrotoxicosis.\(^{122,147}\) These clinical findings of increased glucose levels and insulin resistance in thyrotoxicosis must be kept in mind when patients with both diseases are being assessed. Conversely, insulin requirements are decreased when thyrotoxicosis is corrected. Moreover, diabetic patients with ketoacidosis without a defined precipitating factor need to be checked for thyrotoxicosis. It may not be easy to diagnose thyroid dysfunction in uncontrolled DM as the severe medical condition may impact on the correct interpretation of TH tests. Finally, the pronounced effects of thyrotoxicosis on lipid metabolism may aggravate diabetic heart conditions as will the arrhythmogenic action of elevated TH levels.\(^{110,148,149}\)

### 2.5.2 Subclinical Hypothyroidism

Considering the fact that both subclinical hypothyroidism (SH) and overt hypothyroidism (OH) are frequent co-morbidities in patients with DM, a TSH level determined at diagnosis of diabetes may predict hypothyroidism even at concentrations within the reference range. This is confirmed by a recent retrospective study investigating 1100 patients with DM where baseline TSH concentrations >2.2 mU/l were predictive for subsequent hypothyroidism.\(^{150}\) Hypothyroidism is characterized by impaired glucose absorption from the gastrointestinal tract and delayed peripheral glucose assimilation and gluconeogenesis, decreased or normal hepatic glucose output and decreased peripheral tissue glucose disposal. Moreover, while in OH the inability of insulin to sufficiently sustain glucose utilization by the muscles leads to insulin resistance, SH may also constitute an insulin resistant state. Evidence for insulin
resistance in SH was provided by decreased insulin-stimulated glucose transport rates in isolated monocytes, caused by abnormal translocation of GLUT2, as described earlier.\(^\text{135}\) Glucose disposal is decreased in hypothyroidism, while glucose-stimulated insulin secretion is increased, presumably because of insulin resistance.\(^\text{135, 151}\) A recent clinical study lends credence to this assumption: insulin resistance, tested by short i.v. insulin tolerance test, was induced in euthyroid, thyroidectomized women by acutely stopping T4 replacement therapy.\(^\text{151}\) Direct evidence for a decreased glucose disposal rate, decreased metabolic clearance of glucose and a significantly lowered insulin clearance rate in hypothyroid patients was recently provided. Uptake of oxyglucose on PET in a hypothyroid patient with type A insulin resistance where insulin action is not expected to play any major role was severely impaired during hypothyroidism.\(^\text{152}\) The effect is reversible, as recently shown in a group of 11 patients with OH when rendered euthyroid. As detailed earlier, increased levels of free FA may represent an important cofactor under these conditions.\(^\text{132}\) Insulin resistance, present in both OH and SH, may increase cardiovascular risk, especially when it is associated with other frequently associated risk factors such as hyperlipidemia and elevated blood pressure. Aggressive treatment of these comorbidities, including early correction of SH with TSH serum levels >5 mU/l, seems warranted, particularly with the ongoing discussion of an increased risk of coronary heart disease and mortality in patients with SH.\(^\text{153}\) This is contrasted by a large study of 472 elderly patients with SH and T2DM which did not demonstrate an increase in cardiovascular mortality when compared to a matched group of patients aged over 70 with DM but without SH.\(^\text{154}\) Insulin requirements in hypothyroidism are decreased because of impaired renal insulin clearance. The loss of appetite frequently observed in hypothyroidism may further contribute to a decrease in insulin in underactive thyroid disease. Most importantly, and
explained by decreased gluconeogenesis, hypothyroid patients with T1DM carry a higher risk of hypoglycaemic episodes. As successful correction of hypothyroidism necessitates an increase in insulin dose, associated pituitary or adrenal failure should be considered in these patients.
Figure 2.1. Showing (a) hyper functioning (b) hypo functioning thyroid gland.