Republic of Iraq
Ministry of Higher Education
and Scientific Research
Al-Nahrain University
College of Science
Department of Chemistry

The Profile of Thyroid Hormones, Cortisol, Prolactin and Body Mass Index in Patients with Diabetes Type 2

A Thesis
submitted to the College of Science Al-Nahrain University in partial fulfillment of the requirements for the Degree of Master of Science in Chemistry.

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2011 A.D. **1432A.H.**

Dedication

To

My beloved and dearest man in my life who lighted my way, my father.

To

The kindest woman in my life with most pure endless love in the universe, my mother.

To

The blooming roses in the hardest days of the winter, the joy of my heart, my brothers & sister.

Acknowledgements

This work is not complete without the mention of many individuals who have provided gracious amounts of help and support throughout the research and writing processes. First and foremost, I am thankful for the guidance and leadership of Dr.Salman A.Ali, my advisor. He has provided copious amounts of support and encouragement throughout, while still allowing enough room for my own creative license to steer the research. I am deeply indebted to, and thankful for, pharmacist Bushra J.AL-Tayar, Thualfeqar G. Mohammed and Dr. Mohannad M. Baqir, who have provided access to much of the technical equipment used in this study, as well as extremely beneficial help and thorough methodology, and encourage to keep me precise during the writing of discussions and conclusions. I am also grateful to the staff of the Hormonal Unit at the Central Public Health Laboratory for their help in this study. I am also deeply thankful for the help and support of my friends and family.

Mohammed Al-Najjar

2011

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List of Abbreviations

Abbreviations	Meaning
µL	Microliter
ACTH	Adrenocorticotropic Hormone
BMI	Body Mass Index
dL	decilitre
DM	Diabetes Mellitus
DM2	Diabetes Mellitus type 2
ELFA	Enzyme link fluorescent assay
FPG	Fasting Plasma Glucose
FSH	Follicle Stimulating Hormone
FT4	Free thyroxine
gm	Gram
GH	Growth Hormone
GLUT 4	Glucose transporter type 4
GOD	glucose oxidase
HPA axis	Hypothalamo-Pituitary-Adrenal axis
IDDM	Insulin Dependent Diabetes Mellitus
IFG	Impaired Fasting Glucose
IGT	Impaired glucose tolerance
Kg	Killogram
LH	Luteinizing hormone
m	meter
mg	milligram
mL	milliliter

MODY	Maturity onset diabetes of the young
O.D.	Optical density
OGTT	Oral glucose tolerance test
PRL	Prolactin
SPR	Solid phase receptacle
SPSS	Statistical Package for Social Sciences
T3	Triiodithyonine
T4	Thyroxine
TIDA	Tuberoinfundibulum
TRH	Thyrotropin-releasing hormone
TSH	Thyroid stimulating hormone
Yr	Year

Summery

Diabetes is a group of disorders that produce elevated levels of blood glucose. The two main forms of diabetes are type 1and type 2. The cause of diabetes was associated with some endocrine hormones and obesity. This study was focused on diabetes type 2 and its effects on thyroid hormones (TSH, T3, T4), prolactin and cortisol and with body mass index. To achieve this aim 68 diabetic patients type 2 with ages of 35-70 years and 34 healthy people with ages of 35-70 years (control group) were enrolled. These hormones (TSH, T3, T4, PRL and Cortisol) were estimated by an enzyme immunoassay method with final fluorescent detection (MiniVidas).

The results demonstrated significant increase of T4 ($p<0.05$), Cortisol ($p<0.05$) levels, and significant decrease of TSH ($p<0.05$) levels. A non significant increase was shown in prolactin ($p>0.05$) levels, and non significant decrease in T3 ($p>0.05$) levels.

Obesity is the most important modifiable risk factor in the pathogenesis of type 2 diabetes, however, racial factors seem to be important in the relationship between body mass index and glucose in patients with diabetes type 2.

The linear regression analysis revealed significant negative correlation ($r=-0.310$, $p<0.01$) in cortisol levels with BMI, and significant positive correlation in fasting serum glucose ($r=0.293$, $p<0.05$) and TSH ($r=0.275$, $p<0.05$) levels with BMI in patients with diabetes type 2.

The linear regression analysis revealed significant negative correlation ($r=-0.249$, $p<0.05$) in cortisol levels with fasting serum glucose, and positive correlation in TSH ($r=0.258$, $p<0.05$) levels with fasting serum glucose in patients with diabetes type 2.

The linear regression analysis revealed significant negative correlation ($r=-0.469$, $p<0.05$) in T4 levels with Cortisol levels in patients with diabetes type 2.

Chapter one

Introduction

and

Literature Review

History of diabetes mellitus

Writing about diabetes goes back more than 3000 years, to the Ebers Papyrus, in which afflicted people were described as passing frequent and large amounts of urine. Ayur Veda described the sweetness of the urine and noted that ants were attracted to it. He wrote of weakness, emaciation, polyuria and carbuncles in affected people. Aretaeus, a first – century Greek physician, was credited with naming the disorder. He described the disease as a " melting down of the flesh and limbs into urine ". Lipemia of diabetic blood was noted by Helmunt some time between 1573 and 1664 A.D.. The first diagnostic sign of the disease was established by Thomas Willis in the seventeenth century, when he tasted the urine of his patients and noted its sweetness. A French physician, Michel Chevreul, discovered that the sweetness was caused by sugar.[1]

In 1869, Langerhans, while a medical student, identified the islets but did not understand their function. The discovery of insulin, which began with an accidental observation, when in 1889 Oskar Minkowski and Josef Von Mering, had a friendly disagreement about whether the pancreas, known to contain lipases, was important in fat digestion in dogs. To resolve the issue, they began an experiment on fat digestion. They surgically removed the pancreas from a dog, but before their experiment got any further, Minkowski noticed that the dog was now producing far more urine than normal. Also, the dog's urine had glucose levels far above normal. These findings suggested that lack of some pancreatic product caused diabetes .[2]

The link between islets and diabetes was suggested by De Mayer in 1909 and by Sharpey – Schaffer in 1917, but it was Banting and Best who proved this association in 1921.These investigators used acid – ethanol to extract from the tissue an islet cell factor that had potent hypoglycemic activity. The factor was named " insulin ", and it was quickly learned that bovine and porcine islets

contained insulin that was active in humans. Within a year, insulin was in wide spread use for the treatment of diabetes and proved to be life saving .[3]

1.1 Related Origin of Diabetes Mellitus:-

Diabetes is a family of medical conditions involving the body's ability to metabolize, store, and make use of glucose, which is the main energy source for all cells in the body. This process of converting what we eat and drink into something the body can use requires insulin, a hormone produced by the pancreas. Insulin regulates the conversion of sugars and starches in the blood stream into glucose, and promotes its "uptake" into cells. This is what fuels and energizes cells throughout the body. When a person has diabetes, their body's ability to process glucose is impaired or nonexistent, either because the pancreas does not produce enough insulin or cells have become desensitized to insulin.[3]

1.2 Diabetes Mellitus (DM):-

Diabetes mellitus is a group of diseases characterized by an elevated blood glucose level (hyperglycaemia) resulting from defects in insulin secretion, in insulin action, or both. Diabetes mellitus is not a pathogenic entity but a group of aetiologically different metabolic defects. Common symptoms of diabetes are lethargy from marked hyperglycaemia, polyuria, polydipsia, weight loss, blurred vision and susceptibility to certain infections. Severe hyper- glycaemia may lead to hyperosmolar syndrome and insulin deficiency to life-threatening ketoacidosis. Chronic hyperglycaemia causes long - term damage, dysfunction and failures of various cells, tissues, and organs.[4]

A person with diabetes is more likely to be hospitalized than is a person without diabetes. The hospital stay is also likely to be longer.[5] Diabetes hospitalizations are largely due to the other major health problems caused or worsened by diabetes.[6]

Chronic complications of diabetes mellitus:-

Chronic elevation of blood glucose level leads to damage of blood vessels (angiopathy). The endothelical cells linging the blood vessels take in more glucose than normal, since they don't depend on insulin. They then form more surface glycoprotiens than normal and cause the basement membrane to grow thicker and weaker. In diabetes, the resulting problems are grouped under "microvascular disease" (due to damage to small blood vessels) and "macrovascular disease" (due to damage to the arteries).

The damage to small blood vessels leads to microangiopathy, which can cause on or more of the following:

- Diabetic retinopathy, growth of friable and poor-quality new blood vessels in the retina as well as macular edema (swelling of the macula), which can lead to severe vision loss or blindness.
- Diabetic neuropathy, abnormal and decreased sensation, usually in a 'glove and stocking' distribution starting with the feet but potentially in other nerves, later often fingers and hands.
- Diabetic nephropathy, damage to the kidney which can lead to chronic renal failure, eventually requiring dialysis.
- Diabetic cardiomyopathy, damage to the heart, leading to diastolic dysfunction and eventually heart failure.

Macrovascular disease leads to cardiovascular disease, to which accelerated atherosclerosis is a contributor:

- Coronary artery disease, leading to angina or myocardial infarction ("heart attack").
- Stroke (mainly the ischemic type).
- Peripheral vascular disease, which contributes to intermittent claudication (exertion-related leg and foot pain) as well as diabetic foot.
- Diabetic myonecrosis ('muscle wasting').[7]

1.2.1 Diagnosis of Diabetes Mellitus :-

The National Diabetes Data Group and World Health Organization have issued diagnostic criteria for the diagnosis of diabetes mellitus :

1) Symptoms of diabetes plus random blood glucose concentration ≥11.1 mmol/L (200 mg / dl)[a] or,
2) Fasting plasma glucose ≥ 7. 0 mmol/L (126 mg/dl)[b] or,
3) Two – hour plasma glucose ≥ 11.1 mmol/L (200 mg/dl) during an oral glucose tolerance test [c].

In the absence of unequivocal hyperglycaemia and acute metabolic decompensation, these criteria should be confirmed by repeated testings on different days.[8]

The diagnostic strategy for diabetes mellitus is illustrated in Figure (1.1).[4]

a : Random is defined as without regard to time since the last meal.

b : Fasting is defined as no caloric intake for at least 8 hours.

c : The test should be performed using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water; not recommended for routine clinical use.

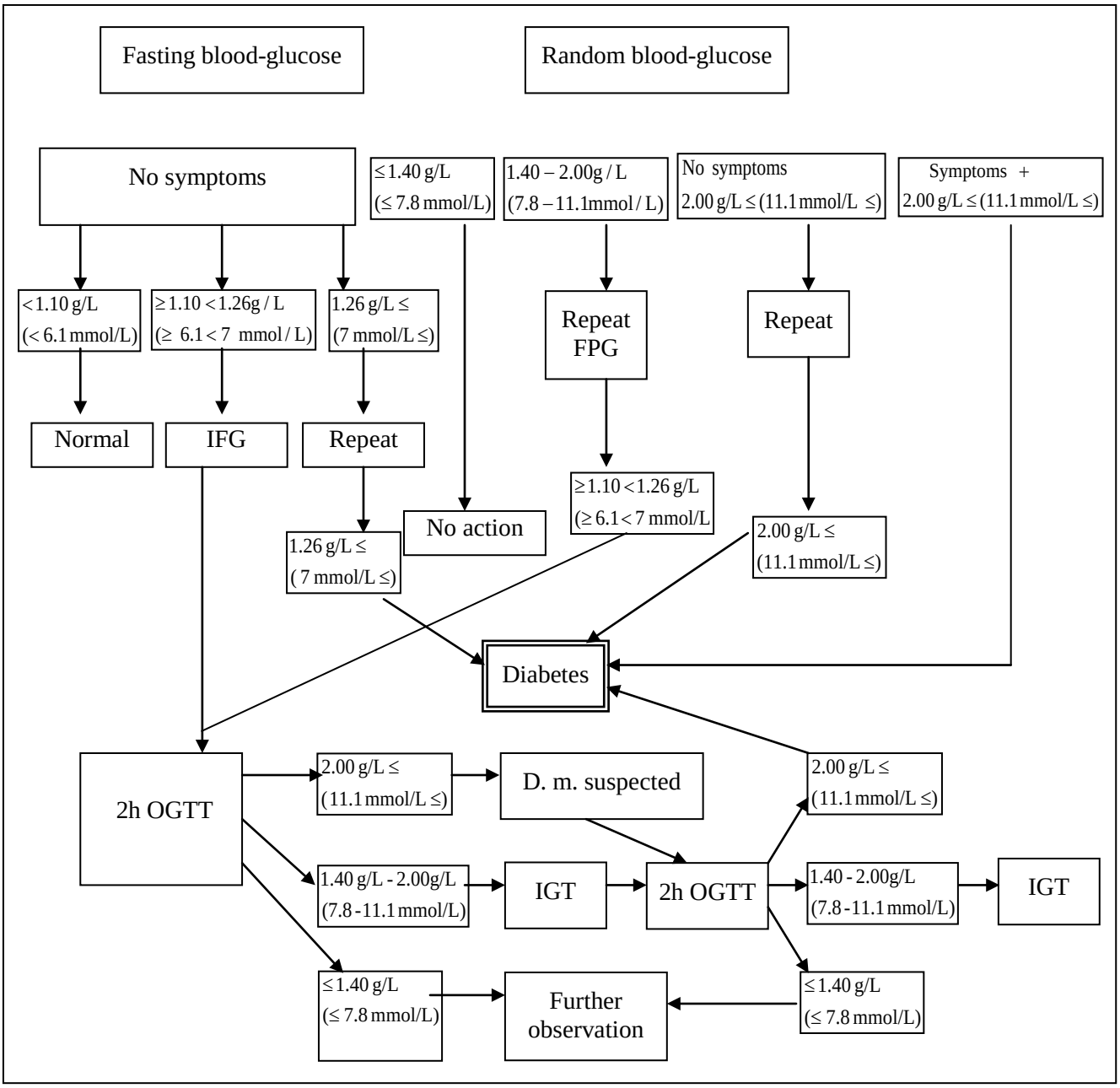


Figure (1.1) : Diagnostic strategy for diabetes mellitus.[4]

1.2.2 Classification of Diabetes Mellitus(DM):-

Diabetes mellitus is a heterogeneous clinical disorder with numerous causes. Two main classifications of diabetes mellitus exist, idiopathic diabetes and secondary diabetes.[9]

Idiopathic diabetes is divided into two main types:

- ❖ Insulin-dependent diabetes mellitus, IDDM (more commonly referred to as type 1 diabetes)
- ❖ Non-insulin-dependent diabetes mellitus, NIDDM (more commonly referred to as type 2 diabetes)

Secondary diabetes, or other specific types of diabetes mellitus are the result of many causes including:

- ❖ Maturity onset type diabetes of the young (MODY) was previously considered to be a third form of type 2 diabetes.
- ❖ Pancreatic disease: Pancreatectomy leads to the clearest example of secondary diabetes.
- ❖ Endocrine disease: Some tumors can produce counter-regulatory hormones that oppose the action of insulin or inhibit insulin secretion.[9]

1.2.3 Type 1 Diabetes Mellitus :-

Type 1 Diabetes mellitus (formerly called type I, IDDM or juvenile diabetes) is characterized by an autoimmune process, usually leading to absolute insulin deficiency. The onset is usually acute, developing over a period of a few days to weeks. Over 95 percent of persons with diabetes mellitus type 1 develop the disease before the age of 25. A family history of diabetes mellitus type 1, gluten enteropathy (celiac disease) or other endocrine disease is often found.[9] However, symptoms appear abruptly when 80% to 90% of the β-cells have been destroyed. At this point the pancreas fails to respond adequately to ingestion of glucose, and insulin therapy is required to restore metabolic control, many patients show a transient disappearance of diabetes and little or no insulin therapy is required. This remission results from a temporary return of insulin secretion, which may last for weeks or months. Ultimately, most patients lose all β-cell function and require insulin therapy.[10]

1.2.4 Type 2 Diabetes Mellitus :-

Type 2 diabetes is a disease that comes from insulin resistance, a condition in which many cells in the body become less responsive to insulin. Before the disease shows clinical signs and symptoms, mildly elevated blood glucose levels can be detected in blood tests, and this stage of the disease is called prediabetes. The progression of type 2 diabetes is gradual. Over the years, a person's prediabetes worsens, especially if the person is overweight and inactive. Eventually, the condition becomes symptomatic and the person develops clinical diabetes.[11]

Insulin resistance is not the only metabolic abnormality in type 2 diabetes. As the disease worsens, pancreatic beta cells begin to fail. At this point, not only is insulin less effective, it is also decreasingly available.[12]

Type 2 diabetes was once called adult onset diabetes because the disease develops slowly and it typically appears in older adults. Ninety to ninty five percent of all cases of diabetes are type 2.[13]

The problems underlying type 2 diabetes include a diminished ability to secrete insulin and a decreased ability of cells to respond to insulin. These problems are difficult to measure directly. Therefore, diabetes is defined indirectly by its effect.[14]

Type 2 diabetes can result from genetics defects that cause both insulin resistance and insulin deficiency. There are two main forms of type 2 diabetes:

1. Late onset associated with obesity.

2. Late onset not associated with obesity.[9]

1.3 Pathogenesis of diabetes type 2

Type 2 diabetes mellitus has become an epidemic, and virtually no physician is without patients who have the disease. Whereas insulin insensitivity is an early phenomenon partly related to obesity, pancreas beta-cell function

declines gradually over time already before the onset of clinical hyperglycaemia. Several mechanisms have been proposed, including increased non esterified fatty acids,inflammatory cutokinase, adipokinase, and mitocondrial dysfunction for insulin resistance, and glucotoxicity, lipotoxicity, and amyloid formation for beta-cell dysfunction. Moreover the disease has a strong genetic component, but only handful of genes have been identified so far: genes for calpain 10, potassium inward-rectifier 6.2, peroxisome proliferator-activated receptor gamma, insulin receptor substrate-1, and other, as shown in figure (1.2).[9]

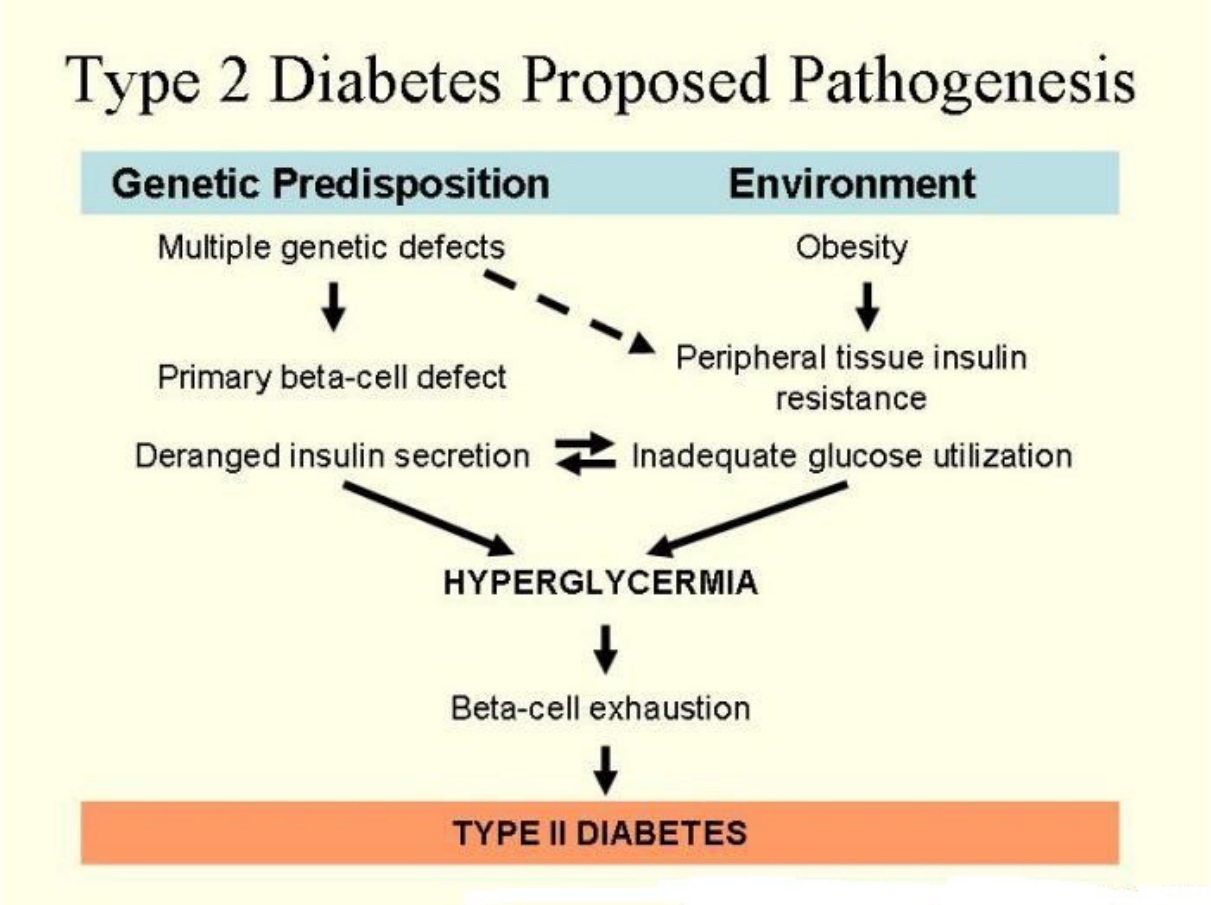


Figure (1.2) pathogenesis of diabetes type 2.[3]

1.4 The predisposing factors , for type 2 diabetes:-

Type 2 diabetes strikes genetically predisposed people, and the tendencies to develop insulin resistance and beta cell dysfunction are inherited. However,

people with these genetic propensities do not always develop clinical diabetes. It appears that, to develop type 2 diabetes, other health problems must intervene to active or to worsen the latent insulin resistance and beta cell dysfunction. [15]

The most important predisposing factors are :

1. A family history of diabetes.
2. Age over 45.
3. Race or ethnic background.
4. Metabolic syndrome.
5. Being overweight.
6. High Blood Pressure and High Cholesterol.
7. History of gestational diabetes.
8. Chronic inflammation.
9. Physical inactivity.

1. A family history of diabetes:

It appears that people who have family members who have been diagnosed with type 2 diabetes are at a greater risk for developing it themselves. There is a stronger inheritance pattern for type 2 diabetes. Those with first-digree relatives with type 2 have a much higher risk of developing type 2, increasing with the number of those relatives. Concordance among monozygotic twins is close to 100%, and about 25% of those with the disease have a family history of diabetes. Genes significantly associated with developing type 2 diabetes, include KCNJ11 (potassium inwardly rectifying channel, subfamily J, member 11), encodes the islet ATP-sensitive potassium channel Kir6.2, and TCF7L2 (transcription factor 7–like 2) regulates proglucagon gene expression and thus the production of glucagon-like peptide-1. Moreover, obesity (which is an independent risk factor for type 2 diabetes) is strongly inherited.[16]

2. Age over 45:

It's a sad but true fact. The older we get, the greater our risk of type 2 diabetes. Even if an elderly person is thin, they still may be predisposed to getting diabetes. Scientists theorize that the pancreas ages right along with us, and doesn't pump insulin as efficiently as it did when we were younger. Also, as our cells age, they become more resistant to insulin as well.[17]

3. Race or ethnic background:

The risk of type 2 diabetes is greater in Hispannics, African-Americans, Native Americans, and Asians.[15]

4. Metabolic syndrome

Metabolic syndrome also called insulin resistance, Twenty years ago, metabolic syndrome was almost an unknown idea among conventional practitioners. Today it is recognized as the precursor to full-blown diabetes. This is gratifying because it means conventional medicine accepts the idea that diabetes develops over time — it doesn’t just appear overnight. The diagnosis of metabolic syndrome is made when three or more of five disorders are present in the patient: high triglycerides, low HDL cholesterol, high blood sugar, high blood pressure and an above-average waistline.[18]

5. Being overweight:

Being overweight is the major contributing factor for type 2 diabetes. About 80% of individuals with type 2 diabetes are overweight or obese, and the risk of type 2 diabetes rises as a person's weight increases. Research suggests that having more fat cells somehow makes cells throughout the body more resistant to insulin.[15]

Results from metabolic and epidemiologic studies provide strong evidence that obesity is causally related to type 2 diabetes. Many studies have

reported associations between body mass index (BMI; in kg/m2) and type2 diabetes in men and women. Investigations focusing on weight change and type 2 diabetes showed that, besides obesity persent, an increase in body weight of 3–20 kg is associated with an elevated risk of incident type 2 diabetes.[19] Early obesity and almost any weight gain after adolescence are risk factors for type 2 diabetes. Moreover, the duration of obesity seems to be a significant risk factor for type 2 diabetes, independently of current degree of obesity.[20]

An inverse linear relation was found between BMI and age at diabetes onset. Adults with early diagnosed diabetes were more obese and more likely to be female than were adults with a later onset of type 2 diabetes.[17]

Although many studies have reported associations between weight gain and risk of type 2 diabetes, no study explicitly quantified whether body weight changes during different periods in adult life are differently related to risk of type 2 diabetes and how much such weight changes influence the age at diabetes diagnosis. This information is urgently needed because a large segment of the population is starting to gain weight early in adult life, after settling into an occupation or family life, which is contributing to the obesity epidemic. The largest increase in the prevalence of obesity in men is seen between ages 20 and 40 y and in women between ages 30 and 40 y. The present investigation examined the effect of body weight gain in early life on the age at diabetes diagnosis and compared 2 different periods in adult life with regard to the association between weight change and diabetes risk. [21]

Any diet that leads to obesity also increases a person's chances of progressing from prediabetes to diabetes. In addition:

- A high-carbohydrate diet stresses the glucose-lowering capacity of a person with prediabetes and accelerates the development of type 2 diabetes.

- A high-fat, low-fiber diet, especially one that includes saturated and trans fats, causes dyslipidemias, and these metabolic imbalances worsen insulin resistance and foster the development of type 2 diabetes.

The composition and calorie content of the diet play an important role in predisposition to type 2 diabetes by reducing insulin sensitivity. Thus a high-saturated fat, high refined carbohydrate and low fiber diet can result in decreased insulin sensitivity , it had been reported that the risk of type 2 diabetes is increased when more than 40% of the total energy intake is from fat.[22]

6. High Blood Pressure and High Cholesterol

These two bad boys are the hallmark risk factors for many diseases and conditions, including type 2 diabetes. Not only do they damage your heart vessels but they are two key components in metabolic syndrome, a cluster of symptoms including obesity, a high fat diet, and lack of exercise. Having metabolic syndrome increases your risk of heart disease, stroke, and diabetes.[17]

7. History of gestational diabetes:

Gestational diabetes affects about 4% of all pregnant women. It begins when hormones from the placenta make the mother insulin resistant. Many women who have gestational diabetes develop type 2 diabetes years later. Their babies are also at some risk for developing diabetes later in life.[17]

8. Chronic inflammation:

Type 2 diabetes alters the functioning of the immune system. Together, insulin resistance, hyperglycemia, and hyperinsulinemia induce a low-level persistent inflammatory reaction. At the same time, a chronic inflammatory state leads to chronic hyperglycemia, which then contributes to the progression of type 2 diabetes.[23]

type 2 diabetes is associated with augmented innate immune function characterized by increased circulating levels of acute phase reactants and altered macrophage biology is fairly well established. The majority investigating innate immune function in type 2 diabetes are limited to the context of wound healing, atherosclerosis, stroke, and other commonly identified comorbidities. Several important recurring themes come out of these data. First, type 2 diabetes is associated with a state of chronic, subclinical inflammation. Second, in macrophages, type 2 diabetic conditions enhance proinflammatory reactions and impair anti-inflammatory responses. Third, after acute activation of the innate immune system in type 2 diabetes, recovery or resolution of inflammation is impaired.[24]

9. Physical inactivity:

Physical inactivity is another major risk factor for the development of type 2 diabetes. In part, this results from the tendency of sedentary people to accumulate triglycerides in their muscle cells. This causes the cells to use fat rather than glucose to produce muscular energy. By way of excess triglycerides, physical inactivity and obesity increase insulin resistance.[25]

Physical exercise is a sufficiently powerful counterweight to insulin resistance that regular exercise will improve glycemic control and reduce the risk of developing cardiovascular complications in people with type 2 diabetes. Furthermore, regular exercise may prevent type 2 diabetes in high-risk individuals.[26]

1.5 Regulation of Blood Glucose in Type 2 Diabetes:-

People with type 2 diabetes have frequent and persistent hyperglycemia. One contributor to this chronic hyperglycemia is the liver. When a person with type 2 diabetes is fasting, the liver secretes too much glucose, and it continues to secrete glucose after the blood level becomes too high. A second contributor to

the chronic hyperglycemia in type 2 diabetes is skeletal muscle. After a meal, the muscles in a person with type 2 diabetes take up too little glucose, leaving blood glucose levels elevated for extended periods.[27]

The metabolic malfunctioning of the liver and skeletal muscles in type 2 diabetes results from a combination of insulin resistance, beta cell dysfunction, excess glucagon, and decreased incretins. These problems develop progressively. Early in the disease—in the prediabetes stage—the existing insulin resistance can be counteracted by excess insulin secretion from the beta cells. The hyperglycemia caused by insulin resistance is met by hyperinsulinemia. Eventually, however, the beta cells begin to fail. Hyperglycemia can no longer be matched by excess insulin secretion, and the person develops clinical diabetes.[28]

1.6 Hormones Involved in Regulating Blood Glucose:

Several hormones are known to control the blood glucose level. The liver plays a vital function in controlling the normal blood glucose level by removing glucose from and adding glucose to the blood. The activity of the liver in maintaining the normal blood glucose level is in turn controlled by several different hormones. Among these are insulin, epinephrine and glucagons, thyroid hormones, growth hormones and glucocorticoids (e.g. cortisol) and also have a definite effect upon carbohydrate metabolism.[29]

Insulin: is a protein hormone produced by the β - cells of the islets of Langerhans in the pancreas.[30] Insulin performs the following functions:

1. It accelerates the oxidation of glucose in the cells.
2. It increases the transformation of glucose to glycogen (glycogenesis) in the muscle and also in the liver.
3. Insulin controls the phosphorylation of glucose to glucose 6- phosphate by means of the enzyme glucokinase.

4. It depresses the production of glucose (glycogenolysis) in the liver.
5. It promotes the formation of fat from glucose.
6. It aids in the transportation of glucose across cell membranes.

Thus, the principle function of insulin may be said to be the removal of glucose from the blood stream and so a consequent lowering of the blood glucose level.[31]

In type 2 diabetes, body cells have a decreased response to insulin. This problem, insulin resistance, means that, for the same amount of circulating insulin, skeletal muscles, liver, and adipose tissue take up and metabolize less glucose than normal. In addition, being less sensitive to insulin, the liver does not react to the "damping" signal of insulin, so the liver manufactures and secretes more glucose than is needed.[32]

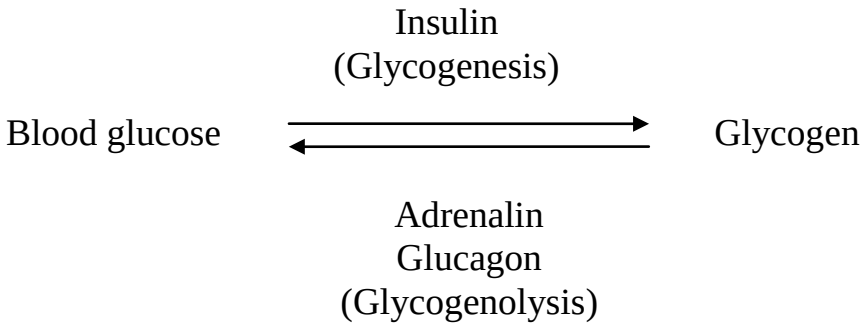
Epinephrine: epinephrine is a hormone secreted by the medulla of the adrenal glands. It stimulates the formation of glucose from glycogen in the liver (glycogenolysis) and so has an action opposite to that of insulin. Insulin removes glucose from the blood stream, whereas epinephrine increases the amount of glucose present in the blood.[33]

During periods of emotional stress, such as anger or fright, adrenalin is secreted by the adrenal glands. It is transported by the blood to the muscles and liver where it promotes glycogen breakdown, thus increasing the amount of glucose in the blood. Making that glucose readily available as the body needs it to meet the emergency situation. The amount of glucose may then exceed the renal threshold (hyperglycemia) and glucose will appear in the urine. This is one example of how the presence of glucose in the urine may be due to a condition other than diabetes.[33]

Glucagon: A hormone secreted by the α–cells of the islets of Langerhans, has several functions that are diametrically apposed to those of insulin. Most important of these is an increase in the blood glucose concentration, an effect which is exactly opposite to that of insulin. Like insulin, glucagon is small

protein it has a molecular weight of 3482 and is composed of a chain of 29 amino acids. On injection of purified glucagons into an animal, a profound hyperglycemic effect occurs. One microgram per kilogram can elevate the blood glucose concentration approximately 20 percent in about 20 minutes. For this reason, glucagon is frequently called a hyperglycemic factor. The two major effects of glucagon on glucose metabolism are (1) breakdown of glycogen "glycogenolysis" and (2) increased gluconeogenesis.[29]

Glucagon thus counter balances the effects of insulin.[34]



Incretins: are glucagon-like peptides, hormones that are made in neuroendocrine cells of the small intestine and are secreted into the circulation in response to food.

Structurally, incretins are closely related to glucagon, but incretins have effects opposite to those of glucagon. Incretins:

- Stimulate insulin secretion
- Suppress glucagon secretion

Incretins are deactivated quickly by enzymes in the bloodstream and by enzymes on the surface of endothelial cells. Therefore, the glucose-lowering effects of incretins last only a few minutes.[35]

People with type 2 diabetes have lower than normal levels of incretins. *Exenatide*, a recently approved injectable anti-diabetes drug, is an incretin-receptor agonist and directly mimics the glucose-lowering effects of natural incretins, and the administration of exenatide helps to reduce blood glucose levels.[36]

Thyroid hormones: Should also be considered as affecting the blood glucose. There is experimental evidence that thyroxin has a diabetogenic action and that thyrodectomy inhibits the development of diabetes. It has also been noted that there is a complete absence of glycogen from the livers of thyrotoxic animals. In humans, the fasting blood glucose is elevated in hyperthyroid patients and lowered in hypothyroid patients. However, hyperthyroid patients apparently utilize glucose at normal or increased rate, hypothyroid patients have decreased ability to utilize glucose. In addition, hypothyroid patients are much less sensitive to insulin than are normal or hyperthyroid individuals.[3]

Growth hormone: Growth hormone inhibits glucose uptake by the tissue. It also inhibits the synthesis of fat from carbohydrate and causes the release of free acid from adipose tissue. Plasma growth hormone normally varies inversely with blood glucose.[37]

Glucocorticoids (e.g. cortisol): These stimulate hepatic gluconeogenesis, and inhibit glucose metabolism in peripheral tissues.[37]

1.7 Pituitary Gland:

Pituitary gland is a pea-sized structure that measures about 1.3 cm in diameter. It is located on the inferior aspect of the brain in the region of the diencephalon and is managed by the hypothalamus which is a section of brain just above pituitary gland. It is attached to the hypothalamus by a stalk like structure called the infundibulum, this contains nerve fibers and small blood vessels.[38]

Anatomically and functionally, Pituitary gland is divided into two distinct lobes, the posterior pituitary and the anterior pituitary .The posterior pituitary differs from anterior pituitary in that the first one stores and releases hormones that are actually produced by the hypothalamus, these hormones are vasopressin and oxytocin, whereas the anterior pituitary produces and secretes its own hormones.[38] The cells of this lobe can be classified simply by their staining reaction as acidophils, basophils and chromophobes. The cells are now classified (by immunochemical means) to at least five cell types, these types are the somatotropes which secrete Growth Hormone (GH) , the lactotropes secrete Prolactin (PRL) , the thyrotropes secrete Thyroid-Stimulating Hormone (TSH) , the gonadotropes secrete Leuteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH) , and the corticotropes secrete Adrenocortico tropic Hormone (ACTH). These hormones are either peptides (ACTH, GH, PRL) or glycoprotiens (TSH, LH, FSH).[39]

1.7.1 Thyroid Hormones

The hormones synthesized and secreted by the thyroid gland are 3, 5, 3', 5'- tetraiodothyronine (thyroxine or T4) and 3, 5, 3'—triiodothyronine (T3). Thyroxine (T4) is the predominant product (~90%) and the biologically active form is T3, which is formed from T4. Synthesis and release of the thyroid hormones are under the control of the hypothalamus-pituitary axis. Thyrotropin releasing hormone (TRH) from the hypothalamus controls the release of thyrotropin stimulating hormone (TSH) from the anterior pituitary. Thyrotropin stimulating hormone promotes synthesis and release of the thyroid hormones by the thyroid gland, as shown in figure (1.3). In order to form the biologically active T3, the almost inactive T4 must undergo enzymatic activation by 5'deiodinases present in different tissues.[40] Most of the thyroid hormones in plasma are protein bound. Aside from their important role in regulation of the

overall rate of body metabolism, thyroid hormones are required for normal brain development during fetal life.[41] Thyroid hormones act by binding to nuclear receptors and modulating the transcription of responsive genes. Fetal thyroid hormone receptors are widely distributed in the brain prior to the onset of fetal thyroid hormone production, and the fetus depends solely on maternal thyroid hormone transfer during this early time period of development. Studies in several species have shown that there is indeed a substantial transfer of maternal thyroid hormones across the placenta until the fetus acquires the ability to synthesize thyroid hormones at the end of the first trimester and contributes to the thyroid hormone supply.[42] Furthermore, the placenta contains deiodinases that convert T4 into the biologically active form T3. Insufficient amounts of thyroid hormones cause abnormal development of almost every organ system resulting in the syndrome "cretinism". The developing brain is especially affected by severe functional deficits leading to mental retardation, ataxia, spasticity, and deafness.[38]

Occasionally, endocrine disorders such as abnormal thyroid hormone levels are found in diabetes.[43] 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. In chemically induced diabetic animals, the alterations in the hypothalamo-pituitary-thyroid axis in diabetic rats are numerous. Hypothalamic and plasma TRH, pituitary and plasma TSH, as well as TSH secretion rate are all reduced, and the TSH response to TRH is decreased despite normal peripheral TSH metabolism. T4 and T3 production and iodide uptake by the thyroid are diminished. 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 deiodination to T3 in peripheral tissues is decreased.[44] The physiological and biochemical interrelationship between insulin and the influence of both insulin and iodothyronines on the metabolism of

carbohydrates, proteins, and lipids are recorded.[45] As Udiong et al. said, "Such records indicate that iodothyronines are insulin antagonists with high levels being diabetogenic, while absence of iodothyronines inhibits the development of diabetes.".[44] Since thyroid hormone abnormalities are frequently associated with diabetes, the present investigation was to assess the adverse effects of diabetes on thyroid hormone levels and other biochemical variables in diabetic patients.[46]

Thyroid disorders are also common in type 2 diabetes because both of these illnesses tend to occur more frequently as people age. Underlying thyroid disorders may go undiagnosed because the common signs and symptoms of thyroid disorders are similar to those for diabetes and can be overlooked or attributed to other medical disorders. Symptoms of hypothyroidism are common in patients with type 2 diabetes and symptoms of hyperthyroidism may be attributed to poor diabetic control in patients with type 1 diabetes.[47]

Because of the link between diabetes and thyroid disease, the American Diabetes Association has recommended that people with diabetes be tested for thyroid disorders. The TSH test, which measures the amount of TSH being produced in the body, is the best test of thyroid function.[47]

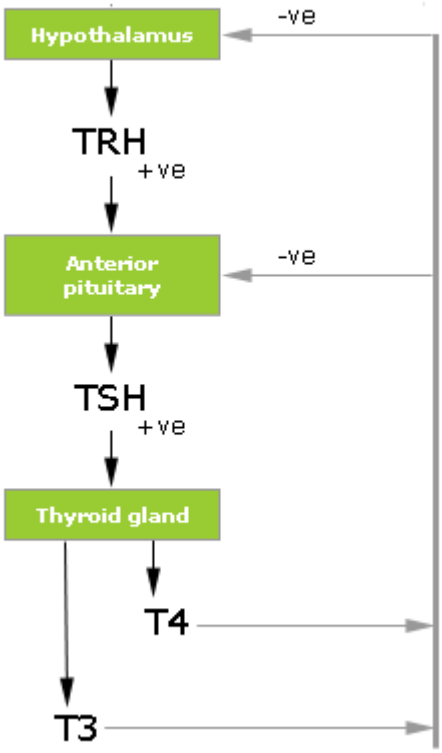


Figure (1.3) Thyroid hormones feedback.[40]

1.7.2 Cortisol

Cortisol, also known as hydrocortisone, is a steroid hormone or glucocorticoid produced by the adrenal gland.[38] It is released in response to stress, and to a low level of blood glucocorticoids. The amount of circulating cortisol is normally subject to a circadian rhythm: the levels of cortisol are highest early in the morning and return to a minimum around midnight.[48] Cortisol has half-life of 60 to 70 minutes; it mainly circulates around (90%) to a carrier protien called transcortin. Corticotropic disorders lead to adrinal hyper or hypofunction, resulting in hyper or hypocorticism. 95% of cortisol in the blood is bound to protein, principally to the cortisol-binding globulin, transcortin.[49]

The secretion of cortisol is controlled by adrenocorticotropic hormone (ACTH); ACTH secretion is subject to feedback inhibition by cortisol. Control

is also exerted from the higher centres through the hypothalamus as shown in figure (1.4). Cortisol is essential to life: it is involved in the response to stress and, with other hormones, regulates many pathways of intermediary metabolism.[39]

Cortisol primary functions are to increase blood sugar through gluconeogenesis, suppress the immune system, and aid in fat, protein, and carbohydrate metabolismIt also decreases bone formation. Various synthetic forms of cortisol are used to treat a variety of different illnesses.[39]

Cortisol exists in serum in two forms. The majority of cortisol is in the bound form, attached to cortisol-binding globulin (transcortin), while the remaining amount of cortisol is in the free, or unbound, form. As the free cortisol leaves the serum to enter cells, the pool of free cortisol in the serum is replenished by cortisol that is released from transcortin or new cortisol that is secreted from the adrenal cortex.[50]

Cortisol counteracts insulin, contributing to hyperglycemia via stimulation of hepatic gluconeogenesis and inhibition of the peripheral utilization of glucose by decreasing the translocation of glucose transporters to the cell membrane, especially GLUT4.[51]

Increasing level of cortisol secretion are related to diabetes complications but did not evaluate central nervous system complications. It has been increasingly recognized that diabetes is related to several neurological and psychiatric disorders. Mild cognitive impairment, Alzheimer's disease, and depression have been associated with diabetes, and these are also related to abnormal cortisol levels. The discovery of how hypercortisolism is related to diabetes complications could help us understand these associations.[52]

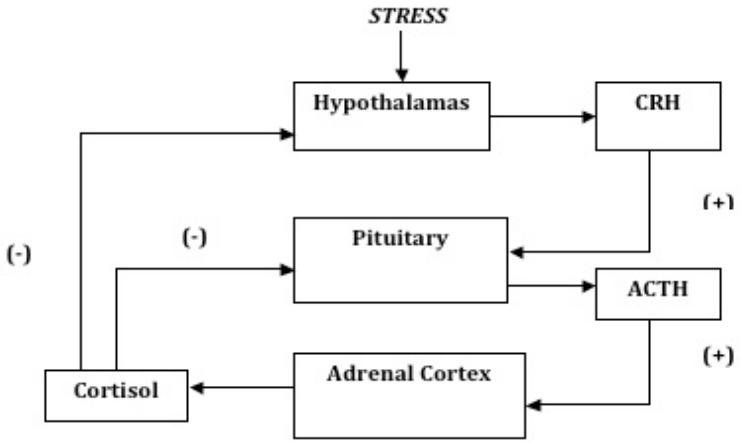


Figure (1.4) Cortisol secretion and feedback. [39]

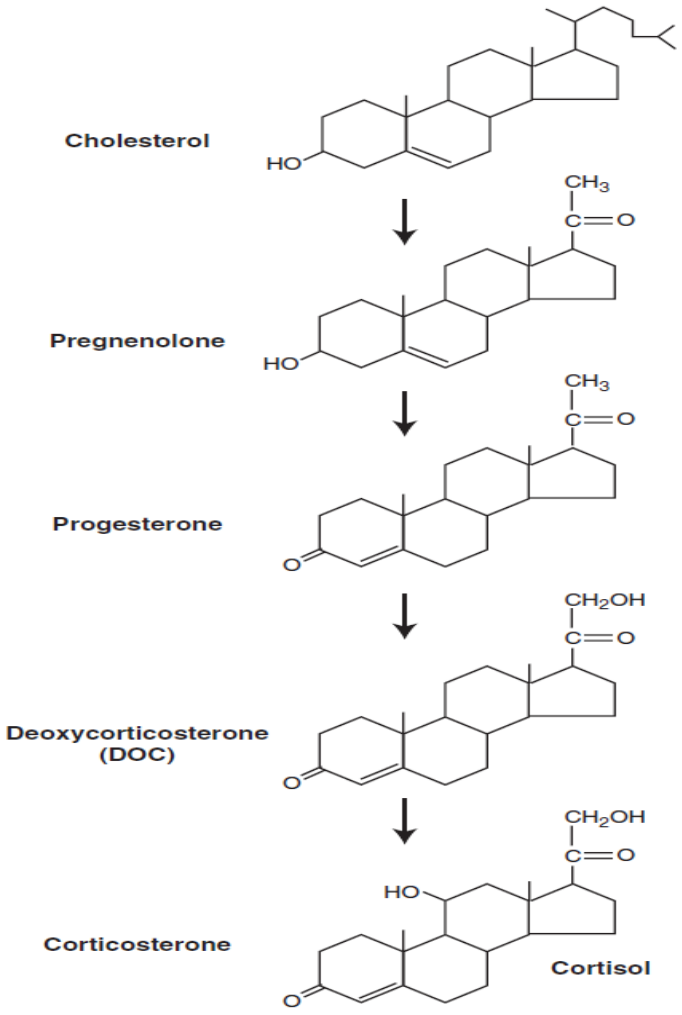


Figure (1.5) Simplified synthetic pathway from cholesterol to cortisol.[3]

1.7.3 Prolactin

Prolactin is a peptide hormone primarily associated with lactation. Prolactin is a polypeptide hormone that is synthesized in and secreted from specialized cells of the anterior pituitary gland, the lactotrophs. The primary role of for prolactin in humans occures during pregnancy when prolactin binds to its receptors in mammary tissue and stimulates the synthesis of several milk proteins, including lacalbumin.[38] Pituitary prolactin secretion is regulated by neuroendocrine neurons in the hypothalamus, the most important ones being the neuro- secretory tuberoinfundibulum (TIDA) neurons of the arcuate nucleus, which secrete dopamine to act on the dopamine-2 receptors of lactotrophs, causing inhibition of prolactin secretion. Thyrotropin-releasing factor (thyrotropin-releasing hormone) has a stimulatory effect on prolactin release. The secretion of prolactin is pulsites, increases during sleep, after meals, exercise and with stress.[53]

Prolactin has been considered a diabetogenic hormone, because injection into dogs results in hyperglycaemia and because endogenous hyper-prolactinaemia in humans is accompanied by glucose intolerance in spite of hyperinsulinaemia . An early study suggested that plasma prolactin was elevated in some diabetic patients, especially those without or with only mild retinopathy.[54]

Somatostatin inhibits the in vitro secretion of prolactin from anterior pituitary cells in monolayer cultures. However, the basal prolactin secretion and the secretion induced by insulin, arginine and TRH in normal man has not been shown to be inhibited by somatostatin . The present study was performed to determine the diurnal plasma prolactin level in normals and juvenile diabetics during 'daily life' conditions, and to investigate the effect of somatostatin infusion on these levels.[55]

Objectives

1. To study the profile of thyroid hormones (TSH, T3, T4) levels in diabetic patients type 2 and comparing with the control group.
2. To study the profile of Cortisol and Prolactin levels in diabetic patients type 2 and comparing with the control group.
3. To study the relationship between thyroid hormones, cortisol and prolactin in diabetic patients type 2.
4. To examine the relevance of several patients related parameters such as age and body mass index in diabetes type 2.

Chapter two

Materials and Methods

2.1 Chemicals

Specific chemicals used in this study are listed in table 2.1 with their suppliers.

Table 2.1The chemicals and companies that supplied them

No.	**Chemical substance**	**Source**
1	VIDAS TSH kit	Biomerieux/France
2	VIDAS T3 kit	Biomerieux/France
3	VIDAS T4 kit	Biomerieux/France
4	VIDAS prolactin kit	Biomerieux/France
5	VIDAS Cortisol kit	Biomerieux/France
6	Glucose kit	Biomaghreb/Tunisia

2.2 Apparatus

Table 2.2 Instruments and their companies that supplied

No.	Apparatus	Source
1	MiniVidas instrument	MiniVIDAS/France
2	UV-Vis Spectrophotometer	Shimadzu UV-Vis/Japan
3	Incubator	Memmert/Germany
4	Centrifuge	Hettich/Germany
5	Oven	Memmert/Germany

2.3 Patients and control groups

The study comprised on 68 diabetic patient (40 male and 28 female). Their ages ranged from 40 – 70 years. The samples were obtained from Al-Kindy

Teaching Hospital/ Specialty Center for Endocrine and Diabetes / Department of chemistry and Central Public Health Laboratory. All the patients were non smokers and not have heart disease and thyriod disease. All measurements were during the period from January 2010 to June 2010.

In addition 34 healthy subjects (20 male and 14 female) were taken as a control group. They did not have any history of chronic disease and did not take any treatment for chronic disease, all control from non smokers, and their ages ranged from 40 – 70 years. The laboratory work was carried out in the Central Public Health Laboratory.

2.4. Collection of samples

Patients should be fast at least 9-12 hours and all the samples collected before 10 am. Disposable syringes and needles were used for blood collection. Blood samples (10mL) were obtained from diabetic patients (patients) and non diabetic (control group) by vein puncture. Samples were allowed to clot at room temeprature and then centrifuged at 3000 Xg for 10 minutes. Sera were devided into two parts one removed and stored at -20˚C until analysis in disposable serum tubes, the other used in the measuring of glucose levels

2.5 Methods

2.5.1 Body Mass Index (BMI)

BMI has been proposed as an alternative to the traditionally used height-weight tables in assessing obesity. BMI measures weight corrected for height and is significantly correlated with total body fat content. BMI was calculated as weight (in kilograms) divided by height (in meters) squared.

BMI= Weight (kg) / Height^2 (m^2)

BMI was classified into :

-Underweight when BMI $<$ 18.5 kg/m^2.

-Normal when BMI between 18.5-24.9 kg/m^2

-Overweight when BMI between 25.0-29.9 kg/m^2.

-Obesity when BMI between 30.0-39.9 kg/m^2.

-Extreme obesity when BMI $\geq$ 40.0 kg/m^2.

2.5.2 Glucose

Principle

Glucose is oxidized by glucose-oxidase to gluconate and hydrogene peroxide according to the following equation

$$\textbf{Glucose} + O_2 \xrightarrow{\textbf{GOD}} H_2O_2 + \textbf{Gluconate}$$

$$2H_2O_2 + \textbf{phenol} + \textbf{4Amino-antipyrine} \xrightarrow{\textbf{Peroxidase}} 4H_2O_2 + \textbf{Quinonimine}$$

Reagent 1 Buffer solution	Tris buffer pH= 7 Phenol	100 mmol/l 0,3 mmol/l
Reagent 2	Glucose oxidase Peroxidase 4-Amino-antipyrine	10 000 U/l 1000 U/l 2,6 mmol/l
Reagent 3	Standard glucose	100 mg/dl 1g/I 5,56 mmol/l

Table 2.3 Reagents of glucose kit

Procedure

Wavelengh : ..... 505 nm

Temerature : ..... 37 °C

Cuvette : ..... 1 cm light path

Zero adjustment : blank reagent

	Blank	Standard	Sample
Standard	----	10 µl	------
Sample	----	-----	10 µl
Working reagent	1.0 ml	1.0 ml	1.0 ml

Table 2.4 procedure of glucose kit

The solutions were mixed at room temperature (20°C -25°C), the color was stable 30 mn. [56]

Calculation

$$\text{Glucose conc. (mg/dl)} = \frac{\text{O.D.Sample}}{\text{O.D.Standard}} \text{ x } 100$$

Normal value : 90-126 mg/dl

2.5.3 Measurement of serum prolactin

Principle

The assay principle combines an enzyme immunoassay sandwich method with a final fluorescent detection (ELFA). The solid phase receptacle (SPR), serves as the solid phase as well as the pipetting device for the assay. Reagents for the assay are ready to use and predispensed in the sealed reagent strips. All the assay steps are preformed automatically by the instrument. The reaction medium is cycled in and out of the SPR severaltimes. The sample is taken and transferred into the well containing the alkaline phosphates-labeled anti-prolactin (conjugate). The sample/conjugate mixture cycled in and out of the SPR several times to increase the reaction speed. The antigen binds to antibodies coated on the SPR and to the conjugate forming a sandwich. Unbound components are eliminated during the washing steps. During the final detection step, the substrate 4-methyl-umbelliferyl

phosphate is cycled in and out the SPR. The conjugate enzyme catalyzes the hydrolysis of the substrate into fluorescent product 4-methyl-umbelliferon. The fluorescence of which is measured at 450 nm. The intensity of the fluorescence is inversely proportional to the concentration of the antigen present in the sample. Results are automatically calculated from the calibration curve stored in the memory, and then printed out .

Procedure

1. Required reagent was taken from the refrigerator and allowed to come to room temperature for at least 30 minutes.
2. One of prolactin strips and one SPR were used for each sample.
3. Prolactin was selected to enter the code and identified by S1and tested in duplicate. The control was also identified by C1.
4. Samples were clarified by centrifugation.
5. The calibrator, control and samples were mixed using vortex- mixer.
6. Two hundred µL of sample, was pipetted into the sample wells.
7. The SPRs and strips were inserted into the instrument .
8. The assay was initiated as directed in the operators manual. All the assay steps were performed automatically by the instrument. The assay was completed within approximately 40 minutes .
9. After the assay was completed, the SPRs and strips were removed from the instrument .
10. The used SPRs and strips were disposed into an appropriate recipient.[57]

2.5.4 Measurement of serum Cortisol

Principle

VIDUS Cortisol S is an automated assay for the VIDUS system, which

enables cortisol in human serum or plasma to be directly quantitatively measured.The assay principle combines an enzyme immunoassay competition method with a final fluorescent detection (ELFA). The solid phase receptacle (SPR), serves as the solid phase as well as the pipetting device for the assay. Reagents for the assay are ready to use and predispensed in the sealed reagent strips. All the assay steps are preformed automatically by the instrument. The reaction medium is cycled in and out of the SPR several times. The sample is transferred into the well containing the conjugate: alkaline phosphatase-labled cortisol derivative. The cortisol in the sample will compete with the cortisol derivative in the conjugate for sites on the specific anti-cortisol antibody which is fixed onto the interior of the SPR. Unbound components are eliminated during the washing steps. During the final detection step, the substrate 4-methyl-umbelliferyl phosphate is cycled in and out the SPR. The conjugate enzyme catalyzes the hydrolysis of the substrate into fluorescent product 4-methyl-umbelliferon. The fluorescence of which is measured at 450 nm. The intensity of the fluorescence is inversely proportional to the concentration of the antigen present in the sample. Results are automatically calculated from the calibration curve stored in the memory, and then printed out .

Procedure

1. Required reagent was taken from the refrigerator and allowed to come to room temperature for at least 30 minutes.
2. One of Cortisol strips and one SPR were used for each sample.
3. Cortisol was selected to enter the code and identified by S1and tested in duplicate. The control was also identified by C1.
4. Samples were clarified by centrifugation.
5. The calibrator, control and samples were mixed using vortex- mixer.

6. 150 µL of sample, was pipetted into the sample wells.
7. The SPRs and strips were inserted into the instrument .
8. The assay was initiated as directed in the operators manual. All the assay steps were performed automatically by the instrument. The assay was completed within approximately 40 minutes .
9. After the assay was completed, the SPRs and strips were removed from the instrument .
10. The used SPRs and strips were disposed into an appropriate recipient.[58]

2.5.5 Measurement of serum thyroid stimulating hormone

Principle

The assay principle combines an enzyme immunoassay sandwich method with a final fluorescent detection (ELFA). The solid phase receptacle (SPR), serves as the solid phase as well as the pipetting device for the assay. Reagents for the assay are ready to use and predispensed in the sealed reagent strips. All the assay steps are preformed automatically by the instrument. The reaction medium is cycled in and out of the SPR severaltimes. The sample is taken and transferred into the well containing the alkaline phosphates-labeled anti-TSH (conjugate). The sample/conjugate mixture cycled in and out of the SPR several times to increase the reaction speed. The antigen binds to antibodies coated on the SPR and to the conjugate forming a sandwich. Unbound components are eliminated during the washing steps. During the final detection step, the substrate 4-methyl-umbelliferyl phosphate is cycled in and out the SPR. The conjugate enzyme catalyzes the hydrolysis of the substrate into fluorescent product 4-methyl-umbelliferon. The fluorescence of which is measured at 450 nm. The intensity of the fluorescence is inversely proportional to the concentration of the antigen present in the sample.

Results are automatically calculated from the calibration curve stored in the memory, and then printed out .

Procedure

1. Required reagent was taken from the refrigerator and allowed to come to room temperature for at least 30 minutes.
2. One of TSH strips and one SPR were used for each sample.
3. TSH was selected to enter the code and identified by S1and tested in duplicate. The control was also identified by C1.
4. The calibrator, control and samples were mixed using vortex- mixer.
5. Two hundred µL of sample, was pipetted into the sample wells.
6. The SPRs and strips were inserted into the instrument .
7. The assay was initiated as directed in the operators manual. All the assay steps were performed automatically by the instrument. The assay was completed within approximately 40 minutes .
8. After the assay was completed, the SPRs and strips were removed from the instrument .
9. The used SPRs and strips were disposed into an appropriate recipient.[59]

2.5.6 Measurement of serum triiodothyronine

Principle

The assay principle combines an enzyme immunoassay competition method with a final fluorescent detection (ELFA). The solid phase receptacle (SPR), serves as the solid phase as well as the pipetting device for the assay. Reagents for the assay are ready to use and predispensed in the sealed reagent strips. All the assay steps are preformed automatically by the instrument. The reaction medium is cycled in and out of the SPR severaltimes. The sample is taken and transferred into

the well containing the T3 antigen labled with alkaline phosphates (conjugate). Competition occures between the antigen present in the sample and the labled anntigen for the specific anti-T3 antibodies coated on the interior of the SPR. Unbound components are eliminated during the washing steps. During the final detection step, the substrate 4-methyl-umbelliferyl phosphate is cycled in and out the SPR. The conjugate enzyme catalyzes the hydrolysis of the substrate into fluorescent product 4-methyl-umbelliferon. The fluorescence of which is measured at 450 nm. The intensity of the fluorescence is inversely proportional to the concentration of the antigen present in the sample. Results are automatically calculated from the calibration curve stored in the memory, and then printed out .

Procedure

1. Required reagent was taken from the refrigerator and allowed to come to room temperature for at least 30 minutes.
2. One of T3 strips and one SPR were used for each sample.
3. T3 was selected to enter the code and identified by S1and tested in triplicate. The control was also identified by C1.
4. Samples were clarified by centrifugation.
5. The calibrator, control and samples were mixed using vortex- mixer.
6. One hundred µL of sample, was pipetted into the sample wells.
7. The SPRs and strips were inserted into the instrument .
8. The assay was initiated as directed in the operators manual. All the assay steps were performed automatically by the instrument. The assay was completed within approximately 40 minutes .
9. After the assay was completed, the SPRs and strips were removed from the instrument .
10. The used SPRs and strips were disposed into an appropriate recipient.[60]

2.5.7 Measurement of serum thyroxine

Principle

The assay principle combines an enzyme immunoassay competition method with a final fluorescent detection (ELFA). The solid phase receptacle (SPR), serves as the solid phase as well as the pipetting device for the assay. Reagents for the assay are ready to use and predispensed in the sealed reagent strips. All the assay steps are preformed automatically by the instrument. The reaction medium is cycled in and out of the SPR severaltimes. The sample is taken and transferred into the well containing the T4 antigen labled with alkaline phosphates (conjugate). Competition occures between the antigen present in the sample and the labled anntigen for the specific anti-T4 antibodies coated on the interior of the SPR. Unbound components are eliminated during the washing steps. During the final detection step, the substrate 4-methyl-umbelliferyl phosphate is cycled in and out the SPR. The conjugate enzyme catalyzes the hydrolysis of the substrate into fluorescent product 4-methyl-umbelliferon. The fluorescence of which is measured at 450 nm. The intensity of the fluorescence is inversely proportional to the concentration of the antigen present in the sample. Results are automatically calculated from the calibration curve stored in the memory, and then printed out .

Procedure

1. Required reagent was taken from the refrigerator and allowed to come to room temperature for at least 30 minutes.
2. One of T4 strips and one SPR were used for each sample.
3. T4 was selected to enter the code and identified by S1and tested in triplicate. The control was also identified by C1.
4. Samples were clarified by centrifugation.

5. The calibrator, control and samples were mixed using vortex- mixer.
6. One hundred µL of sample, was pipetted into the sample wells.
7. The SPRs and strips were inserted into the instrument .
8. The assay was initiated as directed in the operators manual. All the assay steps were performed automatically by the instrument. The assay was completed within approximately 40 minutes .
9. After the assay was completed, the SPRs and strips were removed from the instrument .
10. The used SPRs and strips were disposed into an appropriate recipient.[61]

2.6 Biostatistical Analysis

The data expressed as mean ± SD. Statistical analyses were done by using t-test. Pearson's correlations were also performed. Statistical analysis was performed with the SPSS 16 statistical Package for social sciences and also Excel 2007 with significant difference was set at $P<0.05$.

Chapter three

Results and Discussion

Results and Discussion:

3.1 Serum thyroid hormones, cortisol, prolactin and fasting serum glucose levels in patients and the control group.

Sera of 68 diabetic type 2 patients and 34 healthy person individuals were control group, they were included for the estimation of hormone levels. Results demonstrated significant increase in T4 ($p < 0.05$), and Cortisol ($p<0.05$) levels, and significant decrement in TSH ($p<0.05$), on the other hand the results demonstrated nonsignificant decrement in T3 ($p>0.05$) levels, and nonsignificant enhancement in prolactin ($p>0.05$) levels in diabetic patients type 2 when compared with the control group. Fasting serum glucose levels of diabetic patients type 2 indicated significant enhancement ($p<0.001$) when compared with the control group as shown in table 3.1 and 3.2 .

The evaluation of thyroid hormones level in diabetic patients exhibited low levels of TSH and T3 in association with elevated T4 levels. The most likely attribution for this finding is the decrement in the synthesis of TRH in diabetic patients, and this could be responsible for the occurrence of low thyroid hormone levels in diabetics.[63] This finding show a high incidence of abnormal thyroid hormone levels (high and low) in the diabetic patients. Our observation is in agreement with that reported by Suzuki et al.[63] and Smithson ,[64] who in separate studies found altered thyroid hormone levels of different magnitudes in diabetic patients. The abnormal thyroid hormone levels may be the outcome of the various medications the diabetics were receiving. For example, it is known that insulin, an anabolic hormone, enhances the levels of FT4 while suppresses the levels of T3 by inhibiting hepatic conversion of T4 to T3.[65]

With regard to glucose disposal, the intracellular T3 content in skeletal muscle and white adipose tissue can be considered as an important factor in the

highly regulated transcription/intracellular redistribution of Glucose transporter type 4 (GLUT-4) which result in a decreased intracellular conversion of the prohormone T4 to its active metabolite T3, in secondary individiuals, low intracellular levels of T3 may reduce GLUT-4 transcription, leading to decrease insulin-stimulated glucose disposal. [66-69] A decrease in the circulating T3 levels lead to hepatic gluconeogenesis.[67]

The other explanation of this finding is the non thyroidal diseases such as liver disease and Cushing syndrome where low thyroid hormone levels were recorded.[70,71,72]

The current results demonstrate the promotion of cortisol levels these promotion may be depend on hyperglycemia. It seems probable that the stimulatory effect of cortisol on these variables is a consequence of the direct action of cortisol on hepatic gluconeogenesis.[73] Moreover, in a clinical research center study, Tayek's group also suggested that type 2 diabetic patients might have a mild form of injury response, based on their findings that both type 2 diabetics and cancer patients had; significantly elevated circulating concentrations of cortisol, glucose; depressed triiodothyronine (T3); and elevated glucose production rates. The increased cortisol concentrations were in agreement with Richardson and Tayek[74] they suggest, that individuals with type 2 diabetes may be under minor stress. Increased cortisol secretion is probably also accompanied by increased sympatho-adrenal tone. In patients with type 2 diabetes, counter-regulation is known to start at normoglycemic thresholds, indicating elevated sympathetic neural outflow.[75,76]

Other studies indirectly suggest that cortisol is dysregulated in type 2 diabetics. Atiea and colleagues demonstrated that type 2 diabetics do not undergo the dawn phenomenon,[78] measuring blood cortisol hourly between midnight and 9

AM, this study demonstrated that cortisol levels were significantly higher in newly diagnosed diabetics, especially between 3 and 4 AM. [78]

The elevated levels of prolactin in serum of patients with diabetes occasionally observed hyperprolactinaemia in diabetic patients is not clear. Hyperprolactinaemia is the most common endocrine abnormality of hypothalamo-pituitary axis and prolactin (PRL) is the most commonly hypersecreted hormone by pituitary adenomas.[79] Differences in dietary habits would be one possible explanation since diet is known to influence serum prolactin levels.[80,81,82] An alternative possibility would be abnormalities developing from microvascular infarcts of the pituitary stalk which interfere with the inhibitory stimuli of prolactin secretion. It is noteworthy in this regard to mention that pituitary infarction has been associated with diabetes mellitus.[83]

The high levels of fasting serum glucose in patient with diabetes because Insulin resistance, decreased insulin secretion, and increased hepatic glucose output are the hallmarks of type 2 diabetes.[84]

3.2 Levels of thyroid hormones, cortisol, prolactin and fasting serum glucose in various subgroups of type 2 diabetic patients and the control group

To evaluate serum hormonal levels in various subgroups of type 2 diabetic patients, patients were categorized into three groups according to the results of their BMI analysis. Group 1 consisted of 16 patients with normal BMI**,** group 2 contained 31 patients with overweight BMI**,** group 3 comprised of 21 patients with obese type 2 diabetic patients. The statistical analysis of results was carried out using the ANOVA analysis. The results of TSH, T3, T4, cortisol, prolactin and fasting serum glucose levels, significant ($p<0.05$) increases were indicated for the

levels of T4 and cortisol in the three groups and significant ($p<0.05$) decreases were indicated for the levels of TSH in both overweight and obese groups when compared with the control group. Significant ($p<0.01$) increases were indicated for the levels of fasting serum glucose in three groups when compared with the control group. On the other hand prolactin levels did not show significant variation in the three groups as shown in table 3.3.

In this subgroups we show that the percents of paitients according to their BMI as the following : 23% were normal BMI type 2 diabetic patients, 46% were overweight BMI type 2 diabetic patients, and 31% were obese BMI type 2 diabetic patients.

The fasting serum glucose of all subgroups show highly significant P- value levels, these levels because the insulin resistance and the decrement in insulin secretion.[85]

The levels of TSH shows significant P-value in overweight and obese subgroups only, the evidence of this results may be because the decrement of TRH from the hypothalamus in overweigh and obese diabetic patients that is causes because the increasing the T4 levels that make a negative feedback to the TRH. [86]

The levels of T4 shows significant in all subgroups because the disorders in thyroid gland response to TSH in diabetic type 2 patients, and because T4 not convert to it's active form T3. [87,88]

The levels of corisol shows significant P-value in all groups because the cortisol levels contributed by the CRH from hypothalamus which stimulates by the stress and overweight, there is a link between high cortisol level and obesity by the role of crtisol in the storage of body fat particularly " visceral" abdominal body fat. Visceral fat is particularly unhealthy and cause diabetes. [89,90]

3.3 Correlations of serum thyroid hormones, cortisol, prolactin and fasting serum glucose levels with BMI in patients and the control group.

To verify the correlation of serum thyroid hormones, cortisol, prolactin and fasting serum glucose levels with BMI in diabetic patients, the linear regression analysis was used to analyze the data. The result indicated significant ($r = -0.310$, $P<0.05$) negative correlations for serum cortisol levels with BMI in diabetic patients type 2. Significant positive correlation was obtained for fasting serum glucose ($r=0.293$, $p<0.05$) and TSH ($r=0.275$, $P<0.05$) levels with BMI in patients with diabetes type 2, as shown in table 3.4

BMI showed a positive correlation with fasting serum glucose, Significant increase in mean fasting serum glucose, the mechanism by which obesity induces insulin resistance is poorly understood, but a number of mechanisms have been suspected to be involved. Obesity causes peripheral resistance to insulin-mediated glucose uptake and may also decrease the sensitivity of the beta-cells to glucose.[91] These changes are largely reversed by weight loss, leading to a fall in blood glucose concentrations towards normal levels. Weight gain precedes the onset of diabetes; conversely, weight loss is associated with a decreased risk of type 2 diabetes. [92,93]

A positive correlation between BMI and blood glucose was also reported by other studies. Ethnicity affects the association between obesity and diabetes and that probably explains the different levels of association between obesity and blood glucose levels which are observed in various studies.[94,95,96]

The abnormal values of cortisol in obese diabetic patients with high fasting serum glucose was attributed to the metabolic syndrome is particularly associated with the accumulation of visceral fat.[97] The difference in glucose tolerance despite a similar degree of overweight suggests that the glucose-intolerant subjects might

have been more centrally obese. Noteworthy, it has been demonstrated that visceral obesity is directly related to an impaired cortisone to cortisol conversion.[98]

This results agree with other study (andrews et al) explain the differences in glucose-intolerant subjects compared with normal subjects with matched body mass indices.[99]

A significant and positive correlation was observed between serum level of TSH and BMI in the control group. This results because the insulin influences the hypothalamic-pituitary-thyroid axis, the existant of a reset mechanisim of the central thyrostat in an obese subject is an attractive hypothesis.[100]

The other hormones show nonsignificant levels with BMI in both diabetic and control group.

3.4 Correlations of serum thyroid hormones, cortisol and prolactin levels with fasting serum glucose levels in patients and the control group.

To verify the correlation of serum thyroid hormones, cortisol, prolactin and fasting serum glucose levels with BMI in diabetic patients, the linear regression analysis was used to analyze the data. The result indicated significant ($r = -0.249$, $P<0.05$) negative correlations in cortisol levels with fasting serum glucose of diabetic patients type 2. Significant positive correlation was obtained for TSH ($r=0.258$, $P<0.05$) as shown in table 3.5

Typically, blood glucose levels are unaffected by hypothyroidism because insulin sensitivity is not altered. In fact, in patients utilizing exogenous insulin, there may be a decrease in insulin requirements from reduced insulin degradation.104 Therefore, typically, glucose remains stable or improves while a person is hypothyroid. Once thyroid treatment is initiated, patient education and close observation is vital because normalization of the thyroid may potentially lead

to higher blood glucose levels and loss of diabetes control. In contrast, hyperthyroidism causes insulin resistance and may unmask impaired glucose tolerance and diabetes in previously undiagnosed patients.[101] The significant T4 level with fasting serum glucose in patients groups, may be explained when T4 is the main secretory product of thyroid gland and this show direct role in insulin secretion, or that sub clinical hypothyroidism may be associated with fasting hyperinsulinemia. [102,103]

The significance of cortisol dysregulation is related to the action of cortisol. Cortisol alters blood glucose by affecting glucose transporters in peripheral tissues such as fat and skeletal muscle.[104] Thus cortisol can contribute to elevated blood glucose levels due to inefficient uptake of glucose in the peripheral tissues. While the effects of cortisol on glucose suggest that it may be involved in the cause or progression of type 2 diabetes, there is little clinical evidence to support this theory. However, cortisol has been linked to obesity, specifically central obesity where the excess fat is carried on the midsection of the individual, which is a risk factor for type 2 diabetes.[105-109] Higher levels of cortisol can increase glucose production in the liver, inhibit glycogen synthesis, increase lipid accumulation, and decrease insulin secretion. This combination of events is a probable contributor to the development of type 2 diabetes. [105,110,111,]

Other suggestion of correlation of cortisol is that serum cortisol may influence glucose production. This may be via its ability to stimulate gluconeogenesis. Serum cortisol is directly correlated with the gluconeogenesis in both healthy volunteers and patients with diabetes type 2. [112]

The other hormones show nonsignificant levels with cortisol level in both diabetic and control group.

3.5 Correlation of serum thyroid hormones and prolactin levels with cortisol levels in patients and the control group.

To verify the correlation of serum thyroid hormones and prolactin levels with cortisol in diabetic patients, the linear regression analysis was used to analyze the data. The result indicated significant ($r = - 0.281$, $P<0.05$) negative correlations in T4 serum with cortisol levels of diabetic patients type 2, as shown in table 3.6

The cause of significant T4 with cortisol was not clear but the most likely effective factor is the stress, which is associated with DM2, it may also causes changes in the hypothalamus anterior-pituitary axis in diabetics mellitus type 2. It appears that the presence of subclinical hypothyroidism and hyperthyroidism may result from hypothalamus-hypophyseal-thyroid axis disorders.[103]

The other hormones show nonsignificant levels with cortisol level in both diabetic and control group.

3.6 Correlation of serum thyroid hormones levels with prolactin level in patients and the control group.

All thyroid hormones levels show nonsignificant with prolactin levels in both patient group and the control group as shown in table 3.7

We can consider the relation between prolactin and thyroid hormones by the effects of TRH on both prolactin and TSH (TSH contribute the secretion of T4 and T3). The effect of TRH on prolactin and TSH are reverse, high prolactin levels are found in primary hypothyroidism (decrement of TSH).[113]

Table 3.1 levels of serum thyroid hormonal profile in diabetic patient and the control group

Parameters	Groups	Mean± SD	P-value
TSH (µIU/mL)	*Control *Patient	1.72 ± 0.12 1.59 ± 0.5	0.047
T3 (nmol/L)	Control Patient	1.81 ± 0.62 1.59 ± 0.54	0.084
T4 (nmol/L)	Control Patient	81.37 ± 16.74 90.56 ± 18.34	0.014

***:**Control group NO. = 34

*:Patients group NO. = 68

SD: Standard deviation.

Table 3.2 levels of serum fasting glucose, cortisol and prolactin in diabetic patient and the control group

Parameters	Groups	Mean± SD	P-value
Fasting serum glucose (mg/dL)	*Control *Patient	98.04±24.5 166.33±64.8	0.001
Cortisol (ng/mL)	Control Patient	103.05 ± 34.69 120.49 ± 45.77	0.036
Prolactin (ng/mL)	Control Patient	8.4 ± 4.52 10.26 ± 6.01	0.076

***:**Control group NO. = 34

*:Patients group NO. = 68

SD: Standard deviation.

3.3 ANOVA analysis of thyroid hormones, Cortisol, prolactin and fasting serum glucose data in subgroups of type 2 diabetic patients and the control group

Parameters	**A & B**	**A & C**	**A & D**
TSH	0.058	0.046	0.05
T3	0.082	0.097	0.093
T4	0.016	0.018	0.027
Cort.	0.033	0.047	0.049
PRL	0.19	0.083	0.12
Fasting serum glucose	< 0.001	< 0.001	< 0.001

A : Control group : 34

B : Normal BMI of type 2 diabetic patients group : 16

C: Overweight BMI of type 2 diabetic patients group : 31

D: Obese BMI of type 2 diabetic patients group : 21

Table 3.4 Correlations of serum fasting glucose, thyroid hormons, cortisol and prolactin levels with BMI in diabetic patient and the control group

Parameters	Diabetic patients		Control	
	r	p	r	p
fasting serum glucose	0.293	0.015	0.132	0.626
TSH	0.275	0.023	0.198	0.461
T3	0.181	0.140	0.198	0.463
T4	0.081	0.512	-0.026	0.923
Cortisol	-0.310	0.01	0.317	0.232
Prolactin	0.055	0.670	0.159	0.557

Table 3.5 Correlations of serum thyroid hormons, cortisol and prolactin levels with fasting serum glucose in diabetic patient and the control group

Parameters	Diabetic patients		Control	
	r	p	r	p
TSH	0.258	0.033	-0.368	0.161
T3	-0.146	0.236	0.137	0.612
T4	-0.050	0.685	0.156	0.563
Cortisol	-0.249	0.04	0.151	0.576
Prolactin	-0.183	0.150	-0.165	0.179

Table 3.6 Correlation of serum thyroid hormons and prolactin levels with cortisol levels in diabetic patient and the control group

Parameters	Diabetic patients		Control	
	r	p	r	p
TSH	-0.12	0.329	0.175	0.518
T3	-0.144	0.299	0.457	0.075
T4	-0.281	0.02	0.190	0.480
Prolactin	0.201	0.146	-0.248	0.35

Table 3.7 Correlation of serum thyroid hormons levels with prolactin levels in diabetic patient and the control group

Parameters	Diabetic patients		Control	
	r	p	r	p
TSH	0.048	0.709	0.211	0.0841
T3	0.090	0.485	-0.356	0.176
T4	-0.028	0.829	-0.290	0.277

Conclusion and Future works

Conclusions

1. Diabetic type 2 patients are more susceptible to the development of thyroid hormones dysfunction and cortisol dysfunction.
2. Diabetic type 2 patients are the most vulnerable for hyper prolactinemia.
3. The obese people are the most susceptible to the development of diabetes type 2.

Future works

1. Evaluation of the impact of insulin resistance on the deposition of amyloid in the pancreatic β-cells.
2. The effect of diabetes type 2 on pregnant women.
3. Investigate the effect of diabetes type 2 on kidney's disorders.

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الخلاصة

مرض السكري هو مجموعة من الاضطرابات التي تؤدي الى ارتفاع مستويات السكر في الدم. ويصنف مرض السكري الى قسمين رئيسيين هما داء السكري من النوع الاول وداء السكري من النوع الثاني. وقد اجريت هذه الدراسة على مرضى النوع الثاني فقط ودراسة تأثيراته على مستويات هرمونات الغدة الدرقية والكورتيزول والبرولاكتين وايضا تأثير مؤشر كتلة الجسم. ولتحقيق هذا الهدف فد تمت دراسة 68 شخص مصاب بداء السكري من النوع الثاني تتراوح اعمارهم بين 35-70 سنة و 34 شخص سوي تتراوح اعمارهم بين 35-70 سنة (مجموعة السيطرة).

حيث تم تقدير مستويات تراكيز الهرمون منبه الدرقية (TSH) وهرمون الثيروكسين (T4) وهرمون ثلاثي يودوثيرونين (T3) وهرمون الكورتيزول وهرمون البرولاكتين (PRL) في امصال المرضى بداء السكري من النوع الثاني ومجموعة السيطرة. وايضا تم اخذ طول و وزن جميع الاشخاص المسجلين ضمن هذه الدراسة.

واظهرت النتائج زيادة معنوية ملحوظه في تراكيز هرمون الثيروكسين (T4) ($p<0.05$)وهرمون الكورتيزول ($p<0.05$), بينما لوحظ نقصان معنوي في في مستويات هرمون منبه الدرقية (TSH) ($p<0.05$) لدى الاشخاص المصابين بمرض السكري من النوع الثاني عند مقارنتهم مع مجموعة السيطرة. كما اظهرت النتائج عدم تأثر هرمون ثلاثي يودوثيرونين (T3) ($p>0.05$) وهرمون البرولاكتين (PRL) ($p>0.05$) لدى الاشخاص المصابين بمرض السكري من النوع الثاني عند مقارنتهم مع مجموعة السيطرة. واظهرت النتائج ازدياد السمنة لدى المصابين بداء السكري من النوع الثاني ولهذه الزيادة تأثير على زيادة مستوى السكر في الدم لدى الاشخاص المصابين بمرض السكري من النوع الثاني عند مقارنتهم مع مجموعة السيطرة.

اظهر تحليل الانحدار الخطي ارتباط معنوي سالب لتراكيز هرمون الكورتيزول ($r=-0.310$, $p<0.01$) مع مؤشر كتلة الجسم, بينما ظهر ارتباط معنوي موجب لهرمون منبه الدرقية TSH ($r=0.275$, $p<0.05$)ومستوى السكر في الدم ($r=0.293$, $p<0.05$) مع مؤشر كتلة الجسم لدى مرضى السكري من النوع الثاني, بينما لم تظهر باقي الهرمونات تأثير مع مؤشر كتلة الجسم. كما ظهر وجود ارتباط معنوي سالب لهرمون الكورتيزول ($r=-0.249$, $p<0.05$) مع مستوى سكر الدم, وارتباط مستوى موجب لهرمون منبه الدرقية TSH ($r=0.258$, $p<0.05$) مع مستوى سكر الدم لدى مرضى السكري من النوع الثاني . كما

ظهر ارتباط معنوي سالب بين هرمون الثيروكسين T4 ($r=-0.469, p<0.05$) مع هرمون الكورتيزول لدى مرضى السكري من النوع الثاني.

بسم الله الرحمن الرحيم

(قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا
إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ)

صدق الله العظيم

سورة البقرة الاية (32)

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة النهرين\كلية العلوم
قسم الكيمياء

هرمونات الغدة الدرقية والكورتيزول والبرولاكتين ومؤشر كتلة الجسم لدى مرضى السكري من النوع الثاني

رسالة

مقدمةالى كلية العلوم جامعة النهرين كأستكمال جزئي

لمتطلبات نيل درجة الماجستيرفي الكيمياء

من قبل

محمد صفاء شوكت النجار

بكلوريوس علوم كيمياء 2008

جامعة النهرين

أشراف

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2011 م 1432 هـ